IMPACTS OF POULTRY FARM MANAGEMENT TECHNIQUES ON CONTROL OF *SALMONELLA*

A Dissertation

by

AYOOLA ADEBOWALE ONAFOWOKAN

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Christine Alvarado
Co-Chair of Committee,	James Allen Byrd
Committee Members,	Alejandro Castillo
	Gregory Archer
Head of Department,	Boon Chew

May 2017

Major Subject: Food Science and Technology

ABSTRACT

Despite *Salmonella* control interventions in live poultry production, incidences of poultry *Salmonella* infection have not stopped. These studies evaluated effects of management practices on *Salmonella* transmission in chicken.

Probiotic product was examined in hens. The hens were fed probiotics in ratio 2.6:1 g/ kg of the probiotic to feed and challenged with 10.2 $\log_{10}$ CFU/ 3 mL of antibiotics resistant *Salmonella* Enteritidis 4 times in 6 months. There was no difference between the prevalence and concentration of *Salmonella* in the eggs laid and cecal shedding by either the probiotic fed hens (1.7 % and 2.75 $\log_{10}$ CFU/ g) or control fed birds (2.6 % and 2.95 $\log_{10}$ CFU/ g).

Five out of the twenty-five broiler chicks were orally challenged with antibiotics resistant *Salmonella* Typhimurium and reared in pens lit with either 5 or 50 lux. Blood of the seeder birds was collected and analyzed for leukocyte and heterophil-lymphocyte ratio. There was no difference between the prevalence of *Salmonella* in the contact birds reared under any of the lighting intensities. But the cecal concentration of *Salmonella* was higher in the birds reared under 50 lux ($P = 0.011$). There was no difference between the concentration of leukocyte and heterophil-lymphocyte ratio in the blood of birds raised under either of both light intensities.

Similarly, the impact of rearing birds under either continuous or intermittent lighting from 10 to 20 d was studied. The prevalence and concentration of *Salmonella* was higher in the contact birds reared under continuous lighting ($P = 0.0002$ and > 0.0001

respectively). There was no difference between the leukocyte and heterophil-lymphocyte ratio concentration in the blood of both groups of birds.

Effect of ambient temperature from 2 to 4 wk on *Salmonella* transmission in birds suggested that the prevalence of *Salmonella* was lower in the crops and liver-spleen of contact birds raised in elevated ambient temperature. There was a difference between the indicators of stress in the birds. Birds reared under elevated ambient temperature were significantly stressed in comparison to the birds reared under normal ambient temperature.

Light intensity, scheme and ambient temperature may affect prevalence of *Salmonella* in ceca, crop and liver-spleen of young birds.

DEDICATION

This research work is dedicated to my wife, Akelia and our children, Umm Kulthuum, Zainab, Aisha, Abdusamad and Ameer Onafowokan for their patience and understanding, especially during these research studies.

ACKNOWLEDGEMENTS

I want to give thanks to God Almighty for his mercy and compassion, because without him, all these efforts would have remained a mirage.

I want to extend my gratitude to my supervisor, course advisor and chair of my academic committee, Dr. Christine Alvarado for accepting me into her Laboratory, providing the resources needed for the successful completion of these research works and for mentoring me throughout this program. I would also want to extend my profound gratitude to the co-chair of my academic committee, Dr. James Allen Byrd for allowing me to use his research facilities, providing guidance during planning, designing, implementing and reporting on all of these studies. For without his inestimable supports, all these studies would have remained a dream. I also want to show my appreciation to Dr. Alejandro Castillo for all that I learnt from him throughout my journey in this University, as well as for reading and editing this dissertation. I also want to thank Dr. Gregory Archer for all that I learnt in his classes, especially “The Animal Instinct and Behavior”, for his invaluable contribution towards the completion of all my research studies and for serving on my academic committee.

I would also like to acknowledge the support of Mrs. Denise Caldwell (USDA-ARS), Dr. Kenneth Genovese (USDA-ARS), Rommel Muzo, Amer Hasan, Jiyang Fang, Zahra Hasan, Amie Marie Ibarra Jones, Maria Aguirre, Randi Martin, Emily Kimminau, Dima White, Rekia Adamu, Mahmoud Maaruf, Gerardo Casco and Shailesh Gurung.

Finally, I want to thank my family for their support.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This research work was supervised by a dissertation committee comprise Dr. Christine Alvarado (advisor), Dr. James Allen Byrd (co-advisor) of the Department of Poultry Science, Dr. Alejandro Castillo (committee member) of the Department of Animal Science and Dr. Gregory Archer (committee member) of the Department of Poultry Science.

The bioinformatics data generated in Chapter 3 of this study was analyzed and interpreted in part by Dr. Giri Athrey.

All other works conducted for the dissertation was completed by the student independently.

Funding Sources

There are no outside funding contributions to be acknowledged related to the research and compilation of this dissertation.

NOMENCLATURE

d	Day
wk	Week
h	Hour
min	Minute
yr	Year
CFU	Colony Forming Unit
mL	Milliliter
mol	Mole

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

In the United States, consumption of poultry products has increased over the past ten years (United States Department of Agriculture, 2015a), but there is still concern for the risk of salmonellosis as a result of eating poultry products or contact with live poultry. For instance it was reported that 30 per cent of human foodborne *Salmonella* infection outbreak reported from 2010 to 2015 were due to eating or contact with poultry or poultry products (Centers for Disease Control and Prevention, 2015). Contamination of poultry products with *Salmonella* could occur at any stage of the food supply chain (Bryan and Doyle, 1995). Since live poultry are susceptible to *Salmonella* infection due to many factors, reduction or prevention of poultry products from *Salmonella* contamination will require *Salmonella* intervention at the preharvest level of poultry production (Bailey, 1993; Sanchez, et al., 2002). Mechanisms of poultry infection with *Salmonella* at the farm level have been identified, and results of findings have indicated that one mode of *Salmonella* transmission to poultry at the farm could be through breeders to eggs (Bygrave and Gallagher, 1989; Shivaprasad, et al., 1990; Telzak, et al., 1990). Studies have also indicated that *Salmonella* infection at the farm could also be due to the *Salmonella* contamination of poultry environments (Byrd, et al., 1999; Guard-Petter, 2001; Jones, et al., 1991; Liljebjelke, et al., 2005).

Consequences of foodborne illnesses could be devastating. For instance in the United States the annual illnesses due to foodborne infection were estimated to be 9.4 million and 55,961 patients of foodborne illnesses were hospitalized while 1,351 eventually resulted in death (Scallan, et al., 2011). Furthermore, it was also reported that *Salmonella* accounts for 11 % of the annual foodborne illnesses and *Salmonella* is also implicated in the 35 % of cases of foodborne illnesses that resulted in hospitalization and 28 % of deaths (Scallan, et al., 2011) and the annual cost of foodborne salmonellosis is estimated to be $3.7 billion (United States Department of Agriculture, 2015b).

Since results of studies on contamination of *Salmonella* within the poultry supply chain have indicated that *Salmonella* isolated from processing plants may be from infected live birds (Bailey, et al., 2001; Jones, et al., 1991), control of *Salmonella* at the preharvest level should have a positive impact on the *Salmonella* intervention programs further down the poultry supply chain. For instance, the feeding of a day old chicks with *Salmonella* free ingesta of a healthy adult chicken resulted in increasing the resistance of chicks to *Salmonella* infection, in the study at 1-2 d of age, chicks were orally given 0.5 mL of saline diluted ingesta (1:10 of ingesta collected from crop and intestinal tract) of healthy adult male chickens. Both the ingesta treated chicks and chicks in control groups were orally challenged either low dose (10^3) or high dose (10^6) of *Salmonella* infantis, all the chicks were euthanized between 8 to 22 d of age. Result of the study indicated that 23 per cent or 31 per cent of chicks treated with ingesta tested positive to *Salmonella*, while 100 per cent of the chicks in the control groups were *Salmonella* positive (Nurmi and Rantala, 1973).

Studies on the role of genetic selection have indicated that certain genes may increase poultry resistance to gastrointestinal colonization by *Salmonella*, this was demonstrated in a study where the response of first filial generation of two different sets of chicken breed were assayed when inoculated with *Salmonella* Enteritidis or vaccinated against the pathogen. The location of SNP in CD28 gene was different from that of NRAMP1 gene (Malek, et al., 2004). Other studies have also explored the efficiencies of acidification of poultry feeds and drinking water with coated butyric and propionic acid products in decreasing intestinal colonization by *Salmonella*. Result of the study showed that the level of *Salmonella* presence in the ceca decreased with increase in the concentration of butyric acid in the intestinal tract, also chicken reared on feed and water supplemented with these acids shed lesser *Salmonella* in their feces (Van Immerseel, 2007). Feed supplementation with experimental chlorate product (ECP) has been shown to be effective in controlling *Salmonella* infection in market age birds; 6 wk of age chicken broiler were used in evaluating the efficacies of ECP in controlling *Salmonella* infection. The birds were challenged with *Salmonella* Typhimurium and were divided into 8 treatment groups, which included control feed, control feed + ECP-carrier, control feed with ECP inclusion levels (0.5, 1, 5, 10, 18.5%) and drinking water ECP. Assay of the crop and ceca for *Salmonella* Typhimurium after 7 d of the study indicated that ECP inclusion level of 5 % and above led to significant reduction in the number of birds that tested positive to *Salmonella* when compared to control. However, 5, 10 and 18.5% ECP inclusion level resulted to significant reduction in the level of *Salmonella* colonization in ceca when compared to control group, whereas it was only

10 % inclusion level and water ECP that significantly reduced the level of *Salmonella* colonization in the crop (Byrd, et al., 2008). Prebiotic inclusion in poultry feed is one of the numerous *Salmonella* control interventions that is widely use in the poultry industry. Inclusion of yeast cell wall (4000 ppm) in feed brought about 1.39 log of CFU/ml reduction in the ceca concentration of *Salmonella* Typhimurium in chicks that were orally challenged with the pathogen, also the prevalence of *Salmonella* Dublin was significantly reduced in 10 d old chicks (Spring, et al., 2000a). Furthermore, addition of both normal microbiota and dietary lactose in feed has also offered protection against *Salmonella* infection in broiler chicks, the result of the study showed that inclusion of both probiotics and prebiotics offered additive protection against *Salmonella* infection. Inclusion of dietary lactose resulted to log 2.98 reductions in ceca colonization by *Salmonella* Typhimurium, while normal microbiota inclusion in the feed resulted to log 1.75 in the ceca colonization by the pathogen. And addition of both the culture of the microbiota and dietary lactose brought about decrease of log 4.27 in the population of the pathogen in the ceca (Nisbet, et al., 1993a). The use of antibiotics in poultry is a common method of controlling pathogenic infection, numerous types of antibiotics offer protection against *Salmonella* infection in poultry. Salinomycin, flavophospholipol, polymyxin B, trimethoprim and enrofloxacin are some of the antibiotics that have been shown to protect poultry from *Salmonella* infection (Bolder, et al., 1999; Goodnough and Johnson, 1991; Seo, et al., 2000).

However, efficacies of some of the preharvest measures to control *Salmonella* may depend on meeting certain criteria. For instance, the concept of competitive exclusion

demonstrated by Nurmi and Rantala, 1973, may be effective in chicks that have not been exposed to *Salmonella* at the hatchery (Cox, et al., 1990). Also, despite the effectiveness of antibiotics in control of infectious agents in food animals, the ability of bacteria to develop resistance to antibiotics cannot be overemphasized, research reports have indicated that antibiotics resistant strains of bacteria were isolated from poultry and other livestock (Heuer, et al., 2002; Witte, 2000). The application of prebiotics such as lactose to poultry drinking water may not necessarily transform to *Salmonella* control in broilers (Barnhart, et al., 1999).

Several *Salmonella* control programs have been introduced to reduce incidence of salmonellosis in poultry, more control measures are still needed. Therefore, there is a need to understand the effect of different poultry management techniques on the transmission of *Salmonella* within poultry flocks. For example, lighting management may have impacts on immune response in chicken to antigens, this was demonstrated when two groups of 10 wk of age cockerel that were reared either under constant lighting (CL = 24L:0D) or 12:12D were injected with sheep red blood cell (SRBC) antigen, and were later immunized twice (primary and secondary immunization) against this antigen. Cockerel raised under CL lighting system produced lower antibody titer after secondary immunization against SRBC, they also had delayed hypersensitivity to concanavalin A and phytohemagglutinin (Kirby and Froman, 1991), this study demonstrated that both humoral and cell mediated immune response to infection may be impacted by the lighting system. The choice of the light color used in the poultry rearing may have impact on the immune response of birds to antigen, green and blue lighting

color may aid in increasing immune response while red color lighting depresses immune status (Xie, et al., 2008). Duration of reduced lighting have also been associated with *Salmonella* detection, a multistate study that investigated impact of lighting management on the *Salmonella* infection in a chicken grow out farms indicated that the presence of *Salmonella* on the integument of birds can be associated with the lighting management (Volkova, et al., 2010). Volkova and coworkers reported that birds reared in a farm where daily hour of reduced lighting is greater than 18 h had lower number of birds with *Salmonella* positive integument. Spacing of cages have been shown to have effect on the rate and extent of airborne *Salmonella* transmission among molted egg laying hens, 75 % of birds in adjacent cage to the challenged birds got infected with the pathogen between 3 to 8 d post challenge, whereas only 25 % birds in alternate cages got infected with the pathogen after 10 d (Holt, et al., 1998). Hence, the overall goal of this research work is to investigate how different poultry management techniques, including lighting, heat stress and probiotic inclusion in feed affect *Salmonella* transmission.

Objectives

- To monitor the effect of daily direct fed microbial in feed on the transmission of *Salmonella* in chicken layers
- To determine the effect of different lighting intensities on the horizontal transmission of *Salmonella* in broiler chickens

- To determine the effect of different lighting systems on the horizontal transmission of *Salmonella* in broiler chickens
- To determine the impact of different ambient temperature conditions on the horizontal transmission of *Salmonella* in broilers

CHAPTER II

LITERATURE REVIEW

Brief review on genus *Salmonella*

Salmonella is a member of the family of *Enterobacteriaceae* and like all members of this family, *Salmonella* is a gram negative, non-spore forming, rod shape bacteria that cannot ferment lactose. *Salmonella* is a motile organism, with peritrichous flagella, *Salmonella* is a facultative anaerobe and a mesophilic organism. *Salmonella* is classified into two species: *S. enterica* and *S. bongori*. The *S. enterica* is divided into six subspecies: *S. enterica*, *S. salamae*, *S. houtenae*, *S. arizonae*, *S. diarizonae* and *S. indica* (Popoff, et al., 2000), *Salmonella enterica* is associated with most of the human *Salmonella* infection. *Salmonella* has also been classified for epidemiological purpose base on the infected hosts, Some *Salmonella* serovar cause diseases only in human, which includes *S.* Typhi and *S.* Paratyphi. The second groups are the host adapted serovars like *S.* Gallinarium, *S.* Dublin, *S.* Cholerasuis and the third group is the non-host adapted *Salmonella* serovars. These groups are the zoonotic group and they are the causative agents of human foodborne salmonellosis (Jay, et al., 2005). *Salmonella* has about 2500 serovars, and serovar is named after the location where it was first discovered (Jay, et al., 2005; Popoff, et al., 2000).

Salmonella are intestinal organisms of both human and animal, and presence of *Salmonella* on any other matrices aside intestine of the host is mostly due to fecal

contamination. Food, water, and other materials that are contaminated with *Salmonella* may have had direct or indirect contact with fecal matter. Several food materials have been implicated in the human foodborne salmonellosis outbreaks including poultry and poultry products which are one of the most frequent vehicles of human foodborne *Salmonella* infection (Bryan, 1980; Bryan and Doyle, 1995; Centers for Disease Control and Prevention, 2015). In the past 10 years, poultry commodities were associated with about 26.1 % of illnesses due to foodborne *Salmonella* infection, different serovars of *Salmonella* were responsible for these human poultry borne salmonellosis (Centers for Disease Control and Prevention, 2015).

Investigations of *Salmonella* infection resulting from eating poultry products concluded that poultry products could be contaminated with the pathogen at any stage of the food chain (Bryan and Doyle, 1995). And that minimizing or preventing poultry products contamination with *Salmonella* will require implementation of *Salmonella* control intervention at all the stages of the supply chain. Furthermore investigations have also revealed that some of the serovars of *Salmonella* found at the poultry processing plants were similar to those isolated at the poultry farm (Bhatia, et al., 1979; MacKenzie and Bains, 1976). These reports imply that controlling of *Salmonella* in live birds may reduce the level of poultry carcass contamination with the pathogen in the processing plants. And might reduce the level of *Salmonella* in the poultry products that is delivered to the retails stores and eventually the consumers. Concentration of viable *Salmonella* cells in foods at the point of consumption is very important for establishment of *Salmonella* infection. Other factors that may play a role in the infectiveness of

Salmonella have also been identified and these include the strain of the pathogen, the host immune status, host disease status, age of the host, the composition of the food vehicle of the pathogen and the host physiological status.

Brief review on human foodborne *Salmonella* infection outbreaks due to poultry/ poultry products between years 2010 to 2015 in the United States

Epidemiological studies have suggested that annually about an estimate of 1.2 million sicknesses and 450 deaths in the United States are due to human nontyphoidal *Salmonella* foodborne infection (Scallan, et al., 2011). Also the estimate of the annual cost of human foodborne *Salmonella* infection is $3.7 billion (United States Department of Agriculture, 2015b).

Furthermore reports on the major human foodborne *Salmonella* infection between the years 2010 and 2015 have suggested that 31.6 % of these human foodborne *Salmonella* outbreaks were due to eating/ contact with poultry/ poultry products (Centers for Disease Control and Prevention, 2015). In these outbreaks several *Salmonella* serotypes were implicated, therefore poultry products are carriers of various zoonotic strains of *Salmonella* that are of significant health concerns to human.

Birds could be infected with *Salmonella* in the hatchery, brooding and grow out houses that have been contaminated with *Salmonella* and other pathogens of significance importance to human health (Bailey, et al., 2001; Bailey, 1993; Braden, 2006; Bryan,

1980; Bryan and Doyle, 1995; Byrd, et al., 1999; Cox, et al., 1990; Jones, et al., 1991; Rigby, et al., 1982).

Different serotypes of *Salmonella* have been isolated in the poultry at any of the stages of the poultry production, however the specificity of some *Salmonella* serovars to some particular poultry commodities such as young chicks, broiler, pullets, laying hens and eggs have been suggested (Foley, et al., 2011; FoodNet, 2010; Gast, 2007; Gast, et al., 2014; Keller, et al., 1995). Furthermore, the high incidence of *Salmonella* Enteritidis in laying hens and eggs have been documented (Gast, 2007; Gast and Beard, 1990; Gast, et al., 2004), review on the incidence of *Salmonella* Heidelberg has also suggested that this serovar may be more adapted to laying hens and eggs than some of other *Salmonella* Serovar (Chittick, et al., 2006).

Mechanisms of *Salmonella* infection in poultry

Vertical transmission of *Salmonella*

This is the infection of forming eggs with *Salmonella* as a result of the infection of breeder birds reproductive system. The mechanisms of the pathogen infection of hen reproductive organs include intestine colonized by *Salmonella* that is followed by the phagocytic activities of macrophage that migrate to the reproduction organs and become infected with the pathogen (Gantois, et al., 2009). It was also reported that *Salmonella* in the cloaca of an infected breeder birds may translocate into the lower reproductive organs, colonized these organs and eventually contaminating the descending eggs

(Gantois, et al., 2009). *Salmonella* has been isolated from reproductive organs of both male and female breeder birds (Bygrave and Gallagher, 1989; Gast and Beard, 1990; Gast, et al., 2004).

Numerous studies have revealed that *Salmonella* could be passed on to eggs by the laying hens. Investigation of causative agent of Salmonellosis outbreak due to consumption of egg indicated that total of 5 out of 10 intact eggs tested positive to *Salmonella* and that 2 egg liquids of the egg sampled were positive to the pathogen (Paul and Batchelor, 1988). Reports on the study when laying hens were challenged with four strains of *Salmonella* Heidelberg and one strain of *Salmonella* Enteritidis revealed that all the five strains of *Salmonella* were recovered from egg liquid of the eggs laid by the birds (Gast, et al., 2004). Isolation of the same serotype of *Salmonella* Typhimurium and *Salmonella* Enteritidis from breeder birds and their progeny (Liljebjelke, et al., 2005) have further suggested that forming eggs could be contaminated through infected laying hens. And the chicks that emerged from these eggs may be infected with the pathogen. All these studies point to the possibility of infection of forming eggs by *Salmonella* and its survival in the egg liquid during incubation period.

Other mechanism of poultry *Salmonella* infection due to the pathogen transmission from laying hens to the progenies could also occur by the contamination of intact eggshell and subsequent penetration through pores on egg shell into egg liquid. Egg shell of the formed egg may be exposed to *Salmonella* within the female reproductive system before oviposition or after oviposition due to contact with surface that has been previous contaminated with *Salmonella* (Messens, et al., 2005). *Salmonella* internalization in eggs

may be an important mechanism in young chick infection with the pathogen, since *Salmonella* may survive on egg shell surface for certain period of time after oviposition if egg storage temperature is favorable for the pathogen (Guan, et al., 2006; Schoeni, et al., 1995).

Salmonella cross contamination of egg may be the most frequent mechanism in vertical transmission of *Salmonella* from the laying hen to chicks (Barrow and Lovell, 1991), because in most studies when eggs from *Salmonella* positive laying hen were assayed for the pathogen. The prevalence and concentration of the pathogen in eggs is usually low. In studies where different groups of laying hens were challenged either orally or intravenously with strain of *Salmonella* Enteritidis phage type 4, the eggs and internal organs were *Salmonella* positive few weeks post challenged with the pathogen. The results indicated that there was high prevalence of *Salmonella* in the organs including ovaries and oviducts. *Salmonella* was detected only in 2 out of 633 egg liquids were tested for *Salmonella*. While 36 of the 614 eggs were *Salmonella* positive when the egg shells were tested for the pathogen (Barrow and Lovell, 1991).

This result implied that ovarian or reproductive tract infected with *Salmonella* did not indicate that the yolk or albumen was also infected with the pathogen. Eggs could also be contaminated with *Salmonella* after egg is laid. The study suggested that the main route of egg liquid contamination might be through the egg shell contact with *Salmonella* contaminated surfaces. A similar study indicated that the frequency of *Salmonella* isolated from external egg shells wash water (26.5 %) was significantly higher than the contaminants in the inner egg shell wash water which was 2.9 %

(Bichler, et al., 1996). This study further reinforced the findings of other studies that suggested that the contamination of egg liquid or the inner surface of egg shell during egg formation might not be the main cause of the incidence of egg contamination. Because the prevalence of *Salmonella* in the egg liquid and inner surface of egg shell was lower than the prevalence of the pathogen on egg shell surfaces.

The report of survey of egg laying farm indicated that cloaca swab (4 %), fecal (92 %), egg shell (34 %) were *Salmonella* positive, and that all egg contents tested negative to *Salmonella* (García, et al., 2011). These studies supported the concepts (horizontal transmission of *Salmonella* to eggs) that suggest that there are other mechanisms of *Salmonella* transmission from the laying hen to the chicks other than transovarian transmission of *Salmonella* to the forming eggs. The mechanism of horizontal transmission of *Salmonella* to the egg during or after oviposition is further supported by the studies that demonstrated the translocation of *Salmonella* Typhimurium and *Salmonella* Enteritidis from the external egg shell surfaces to the internal egg shell and its component, especially in the freshly laid eggs (Miyamoto, et al., 1998).

Penetration of *Salmonella* into internal egg content was also described in a study in which the egg shell inner surface was filled with microbial culture agar, and the agar was observed for the growth of *Salmonella*. The rate of *Salmonella* penetration into the internal content of the egg shell was positively affected by the concentration of the pathogen on the egg shell external surface and the storage conditions (Messens, et al., 2006; Schoeni, et al., 1995). *Salmonella* penetration from the egg shell surface into the egg contents was enhanced when the eggs inoculated with *Salmonella* were exposed to

the incubation conditions that were similar the incubation conditions in the hatchery (Schoeni, et al., 1995).

However, irrespective of the mechanisms of the *Salmonella* transmission from the laying hens to eggs, the prevalence of *Salmonella* infection in chicks that emerged from eggs that were contaminated with *Salmonella* is high. Also the part of the eggs (either the egg shell or egg liquid) contaminated with *Salmonella* might not have effect on the prevalence of *Salmonella* in chicks during hatching. Therefore, it is very important to protect eggs from exposure to *Salmonella* contaminated contact surfaces during egg formation and before or after oviposition, since this might prevent the incidence of vertical transmission of *Salmonella* to the young chicks.

Several works have been done on investigating different characteristics of egg shell that could have effect on bacterial penetration from the external egg shell surfaces into internal egg surfaces. While some studies have attributed bacterial penetration to the location of the bacteria on the contaminated shell to the features of the egg shell such as the thickness of the shell, the density of the shell egg, the amount of the pores on the shell or age of the laying hens that laid the egg. Findings from a study that used the egg shell agar filled and whole intact egg approaches suggested that cuticle characteristic of the egg affected the rate of bacterial penetration into the inner content of egg (De Reu, et al., 2006). The study indicated that the rate of *Salmonella* translocation from the outer egg shell surface to the egg liquid increased with lower concentration of cuticle covering the egg. The strain of the bacterial on the egg surface might also determine the ability of the bacterial to penetrate into the inner content of intact eggs. And *Salmonella* Enteritidis

had been identified as one of the bacteria strain that possessed the ability to translocate from the external shell egg surface into internal content of egg (De Reu, et al., 2006).

Controlling vertical transmission of *Salmonella* in poultry should include strategy that will increase resistance of breeder birds to *Salmonella* infection. And the effective sanitation program that will reduce or eliminate the exposure of breeder birds to the pathogen in the environment. Because *Salmonella* free breeder birds will most likely lay eggs that are not contaminated with *Salmonella.*

Horizontal transmission of *Salmonella*

Salmonella is an intestinal organism of human and animal but could also be found on the other part of human and animal body parts, mainly due to contact with fecal matter. The main mode of *Salmonella* transmission to none infected bird is fecal oral route, although other routes of *Salmonella* infection have been suggested in poultry (Gantois, et al., 2009; Gast, et al., 1998; Harbaugh, et al., 2006; Nakamura, et al., 1997). Therefore, environmental agents that might have had direct/ indirect contact with fecal matter may become contaminated with *Salmonella,* hence serve as agents of *Salmonella* transmission from a *Salmonella* infected poultry to non - *Salmonella* infected poultry in the same flock. Poultry environmental agents that have been associated with *Salmonella* are discussed in the subsequent paragraphs.

Contact between poultry

Cross infection between poultry is probably one of the most important agents of *Salmonella* transmission among poultry of the same flock. *Salmonella* are intestinal organisms of poultry and are frequently isolated in the cecal and fecal matter of infected birds. Presence of few *Salmonella* infected poultry in the flock during stocking might result to infection of a large per cent of the birds during growing period (Rigby and Pettit, 1979; Snoeyenbos, et al., 1969). This suggested that maintaining *Salmonella* free flock, efforts should be made to ensure that none of the incoming chicks is a carrier of the pathogen. And it is important to follow proper hygienic procedures before placement of new stocks, since infectious fecal matter of the previous flock may serve as agent of *Salmonella* infection to the new flocks (Marin, et al., 2011). Bird could be infected with various serotype of *Salmonella* (Snoeyenbos, et al., 1969) without showing any symptom of infection except in the case of host adapted serotypes such as *Salmonella* Gallinarium and *Salmonella* Pullorum (Jay, et al., 2005). Experimental studies have also suggested that the presence of as few as 2 *Salmonella* infected birds in a pen could result to the infection of all or all the penmates (Snoeyenbos, et al., 1969).

Several factors may play a role in the transmission of *Salmonella* among poultry housed in the same pen, one of which may be the extent of the motor activities of the birds in the pen, and this can be influenced by lighting management (Volkova, et al., 2010). Lighting characteristics in the pens could be a factor in the transmission of *Salmonella*, because birds motor activities may be affected by photoperiod and light intensity (Blatchford, et al., 2009; Martin, 1989; Newberry, et al., 1988; Simmons,

1982). Birds reared in farms with lighting scheme that enhanced high motor activities may have higher prevalence of *Salmonella* (Volkova, et al., 2010). High environmental temperature in the poultry house might induce heat stress in birds. And an increase in the fecal shedding of *Salmonella* in heat stressed birds had been reported (Burkholder, et al., 2008; Soerjadi, et al., 1979; Traub-Dargatz, et al., 2006). Heat stress could also result in decrease in the immune response status (Dietert, et al., 1994; Quinteiro-Filho, et al., 2010), therefore heat stress condition could lead to increase in the prevalence of the pathogen in the environment, while also lowering the resistance of bird to *Salmonella* infection.

Interventions to increase the resistance of chicken to *Salmonella* infection have been developed; vaccination, competitive exclusion, genetic selection, modification of feed (with probiotics, prebiotics and synbiotics), antibiotics, feeding with chlorate, feed and drinking water acidification and other strategies have been extensively studied. And some of these *Salmonella* resistant strategies had been applied in solving economically significant poultry *Salmonella* infection challenges of the past, such as elimination of *Salmonella* Gallinarium in most parts of the world. And majority of the strategy are still applicable in modern poultry industry. It should be noted that control of horizontal transmission of *Salmonella* infection in poultry through control of the pathogen in the environment is extremely important. However, increasing the resistance of poultry to *Salmonella* infection could also be an important control strategy. Therefore, combination of both the sanitation and increasing the resistance of bird to *Salmonella* infection will bring about a desirable result in control of the pathogen in poultry industry.

Feed

Result of survey of feed mill suggested that feed mills are one of the vehicles of *Salmonella* infection in the poultry. In a field survey of ingredients (meat and bone) collected and tested for the presence of *Salmonella* suggested that 60 % of meat and bone meal sampled were *Salmonella* positive (Jones, et al., 1991). Also 35 % of mash feed were contaminated with the pathogen. However there was 82 % reduction in the prevalence of *Salmonella* in the feed after pelleting process suggesting that pelleting the meal may have lower incidence of *Salmonella* when compare to the mash feed (Jones, et al., 1991).

In another study where samples of different feed ingredients, processing feed and the final feed products were tested for the presence of *Salmonella*, the result of the study also suggested that feed might play a role in chicken infection with *Salmonella*. Five of eleven categories of feed ingredients samples were positive for *Salmonella* (Jones and Richardson, 2004). The study suggested that *Salmonella* contamination could occur at any stage of feed production. Some samples were positive even after pelleting and the final feed products were contaminated with *Salmonella* (Jones and Richardson, 2004). These results implied that *Salmonella* positive feed ingredients might have been eliminated or reduced during pelleting but recontaminated by the contact surfaces and other *Salmonella* contaminated agents in the feed mill.

Apart from the poultry feed and its ingredients, other agents either biotic agent such as insects and rodents (Jones, et al., 1991; MacKenzie and Bains, 1976) or abiotic factor like dust (Jones and Richardson, 2004) are *Salmonella* carriers. Even if feed milling

operations such as pelleting and cooling were able to inactivate *Salmonella* in the feed, post process contamination of feed, packaging material due to contacts with the biotic and abiotic agents may still occur. Therefore, the importance feed in poultry infection with *Salmonella* cannot be overemphasized and efforts to control *Salmonella* in poultry will yield more results if more attention is being paid to *Salmonella* control at feed mills. Other studies on the prevalence of *Salmonella* in feed mill environment, feed processing machines and equipment have also suggested that final feed products may remain *Salmonella* contaminated even if the ingredients used for feed production are free of pathogen (Davies, et al., 2001). Most of the samples tested in the study were contaminated with *Salmonella*, suggesting that the equipment may be harboring and introducing contaminants to feed during processing.

Apart from the contaminants that may be present in the feed prior to been supplied to poultry farms, feed from feeder are frequently contaminated with *Salmonella* by other *Salmonella* carriers at the farm. Hence may serve as vehicle of infection to uninfected poultry. Samples of feed from feeders collected during grow out period in the survey of 65 broiler farms in a region of Spain indicated that the prevalence of *Salmonella* in the samples was 16 % (Marin, et al., 2011).

Investigation on the persistence of *Salmonella* in poultry farms has suggested that by the end of each growing cycle the feeding trough should be cleaned and disinfected. Strains of *Salmonella* have been found in the poultry rearing site, laying houses and hatchery several months after the poultry reared in them have been depopulated due to prevalence of *Salmonella* in the flock (Davies and Breslin, 2003b). The persistence of

Salmonella in the environment has also been reported in other studies, especially when factors such as adequate nutrient, moisture, pH, temperature and oxidation-reduction required for microorganism growth, survival and proliferation are favorable (Jay, et al., 2005). *Salmonella* and few other gram negative bacteria are not as fastidious as most gram positive bacteria (Jay, et al., 2005) and can survive in the environment even if required growth factors are limited. Feed contaminated with *Salmonella* might not result in *Salmonella* infection in live poultry only, but might be further transmitted to human.

Water

Studies on the effects of environmental factors as vehicle of *Salmonella* infection have suggested that the contaminated water drinker might be a vehicle of *Salmonella* transmission in poultry flocks (Davies and Breslin, 2003b; Nayak, et al., 2003). *Salmonella* contaminated water or water drinkers will probably be an agent of *Salmonella* transmission among poultry flocks in the same poultry pen by cross contamination. Dust, insects, rodents, *Salmonella* infected poultry and some other potential carriers of *Salmonella* might contaminate water and/or water supply system.

Litter and chicks tray liner

Contaminated litter may act as *Salmonella* transmission agent to poultry (Davies and Breslin, 2003b; Fanelli, et al., 1970; Kinde, et al., 2005; Lapuz, et al., 2008; Rigby and Pettit, 1979). Similarly, tray liners used for chicks transportation from the hatcheries to

the grow out house may contribute to horizontal transmission of *Salmonella* in the young poultry (Marin, et al., 2011).

Pine wood spread on concrete floor was shown to be an important agent in the spread and maintenance of *Salmonella* Typhimurium infection in birds irrespective of the age of the litter (Rigby and Pettit, 1979). During the 3 trials of studies reported by Rigby and Petit (1979), it was shown that the litter acted as the main agents of *Salmonella* infection to 2 different *Salmonella* free flocks of birds placed on it. The new litter became *Salmonella* positive on 3 d of the study and remained contaminated until the end of the 3 studies (163 d). Although the *Salmonella* load of the litter decreased gradually from 7 $\log_{10}$ CFU/g on 30 d to 2 $\log_{10}$ CFU/g on 163 d. In another study that compared the *Salmonella* infective rate in the birds reared on litter to those in cage indicated that by 38 d of the study, *Salmonella* shedding in the fecal of the birds on litter was at least 5 $\log_{10}$ CFU/g whereas those of the birds in cage was between 1 to 2 $\log_{10}$ CFU/g (Rigby and Pettit, 1979).

Numerous reports are in agreement about the persistence of *Salmonella* in the litter and the role of litter as an agent of *Salmonella* transmission, the freshness of the litter may be playing additional role in the *Salmonella* infective and colonization rate of birds (Fanelli, et al., 1970). Fanelli, et al. (1970) reported that birds reared on the built up litter were *Salmonella* positive on 28 d and 35 d respectively, while 65 % and 41 % of birds reared on fresh litter tested positive to the pathogen on 28 d and 35 d respectively. This report suggested that the age of the litter had influence on the survival and the growth of *Salmonella*. Also after the removal of birds from both sites, *Salmonella* was isolated on

the built up litter through 49 d and on the 63 d, whereas the pathogen was isolated on the fresh litter up to 56 d and on 70 d and 91 d (Fanelli, et al., 1970). The study further revealed that *Salmonella* may be able to persist longer in fresh litter than in the older ones. The reason for the disparity in the behavior and survival of *Salmonella* on litters of different ages might be due to certain extrinsic factors such as water activity (a_w), pH and nutrient.

The growth and survival of microorganisms depends on the extrinsic properties of the growth medium (Jay, et al., 2005), the age of pine wood shaving used may have effect on it a_w, pH and probably on the nutrient availability to bacteria. Ambient temperature in the poultry houses usually ranges from 33 °C to 21 °C depending on the breed and age of the chicken. Exposure of litters to these temperatures will induce evaporation of moisture in the litters and the duration of litter in the poultry houses will definitely affect the quantity of its water content and this may have impact on the a_w of the bacteria. The pH of fecal matter of poultry is low due to the presence of uric acid, the metabolite of amino acids, also the pH of the decomposing feed might be low, therefore the pH of the litter may be impacted by the fecal matter and decomposing feed and resulted in antagonistic effect on the pathogen. Also medium with a lowered a_w and pH, the nutrient uptake of bacteria will be low hence the growth the bacteria in the such medium will be negatively affected (Jay, et al., 2005). Therefore, in the built up litters the pH, nutrient and a_w might be lower than in the fresh litter, hence the built up litters might provide an inhibitory condition for survival of *Salmonella* in the poultry houses.

Other environmental agents of* Salmonella *transmission in poultry

Insects could be carriers of *Salmonella* in both poultry houses and feed mills (Davies and Breslin, 2003a; Dewaele, et al., 2012; Jones, et al., 1991; Kinde, et al., 2005; Kopanic, et al., 1994). For example, a trace back study on the sources of a multistate human poultry borne *Salmonella* infection outbreak in the United States between July 2012 to February 2013 indicated that crickets were the agents of *Salmonella* infection to the hatchery where the implicated chicks were purchased from (Nakao, et al., 2015). Rodents are also one of the agents of *Salmonella* infection in chicken (Davies and Breslin, 2003b; Dewaele, et al., 2012; Lapuz, et al., 2008). Investigation of some egg layer farms identified as producers of *Salmonella* positive eggs have indicated that rodent infestation was one of the factors responsible for *Salmonella* dispersal in the farms and that the level of rodent presence on the farm was directly related to the level of *Salmonella* contamination of eggs in the farm (Carrique-Mas, et al., 2009).
Air has been identified as one of the *Salmonella* dispersing agent in poultry farms (Cason, et al., 1994; Gast, et al., 1998; Kallapura, et al., 2014a; Kallapura, et al., 2014b). The direction of the airflow might not influence the rate of *Salmonella* transmission among birds (Nakamura, et al., 1997).

Dust generated in the poultry houses could be one of the agents of *Salmonella* dispersion in poultry houses (Davies and Breslin, 2003b; Harbaugh, et al., 2006; Marin, et al., 2011). Survey has shown that dust control in the poultry house might be required to effectively control prevalence of *Salmonella* in poultry. Investigation into the factors that was implicated in the prevalence of *Salmonella* at the production stage of poultry

supply suggested that 25 % of dust samples collected from 65 farms in Spain contained *Salmonella* (Marin, et al., 2011). Also 15 % of the contact surfaces sampled were *Salmonella* positive (Marin, et al., 2011).

Farm workers have also been implicated in the poultry *Salmonella* infection, humans might be enteric carrier of the pathogen, but the isolation of the pathogen from the hands of poultry worker supported the possibility that humans could be agents of poultry infection with the pathogen (Yhiler and Bassey, 2015).

Salmonella control strategies at the preharvest level of poultry (chicken) production

Sanitation and personnel training

The best strategy for *Salmonella* control in the poultry production is the prevention of the poultry farm contamination with *Salmonella.* Therefore, efforts should be made to ensure that whatever object that will be entering poultry farm should be free of *Salmonella*, and this will start with feed, water, litter and all over materials that are needed in the rearing of poultry. Pest control should also form the integral part of *Salmonella* control strategy, since all pests have been identified as capable of contaminating feed, water and contact surfaces with *Salmonella* (Davies and Breslin, 2003b; Dewaele, et al., 2012; Holt, et al., 2007; Jones, et al., 1991). Furthermore, concerted efforts should be made not to purchase birds from breeders or hatcheries that have history of supplying Salmonella infected birds.

And poultry management team should ensure that all Good Manufacturing Practices (GMP), Good Agricultural Practices (GAP) and other Standard Sanitary Procedure

(SSP) are strictly adhered in the farm. Furthermore, all employees should be adequately trained on how to care for the animals.

Vaccination

The concept of vaccination involves introduction of a known antigen to a host with the purpose of stimulating the host immune response to a given organism. And this will quicken and increase the antibody secretion in the host in case infection with bacteria that carry same antigen as antigen contained in the vaccine.

Types of vaccines

Vaccines that have been tested and demonstrated to have impact on *Salmonella* spp. include; live-attenuated, whole-killed / Inactivated (Woodward, et al., 2002), subunits and genetically modified mutant vaccines.

Killed vaccines. These have been used to control non-host specific *Salmonella* infection in poultry with different outcomes (Barrow, et al., 1990; Davison, et al., 1999; Gast, et al., 1992). Inactivation of the bacterial is achieved by either heat or formalin application. Reports on studies have suggested that inactivated vaccines can only produce humoral response which can aid in bacterial shedding reduction (Arnon, et al., 1983; Babu, et al., 2004; Collins, 1972). Since cell-mediated immune response might be having more impact in tissue clearance of *Salmonella*. Therefore, inactivated vaccines may be limited in eliciting optimal immune protection in poultry against *Salmonella* Enteritidis. Field and experimental challenges has reported varied results on the ability

of bacterins to decrease organ colonization and fecal shedding of *Salmonella*. Further, process of inactivation, type of adjuvant and method of culturing bacteria used in vaccine preparation may affect the efficacy of the vaccine (Barbour, et al., 1993; Nakamura, et al., 1994b).

Study on the protection of laying with vaccines which comprised of inactivated *Salmonella* Enteritidis cells in oil emulsion media was administered to birds at both 8 and 16 wk (booster dose) of age. At 20, 25 and 31 wk of age, the birds were orally challenged with 2.13×10^9 CFU/mL of *Salmonella* Enteritidis. Spleen, liver, ovary and cecal contents of the birds were assayed for *Salmonella* 2 d after been challenged, also cloacal swab and eggs were assayed for *Salmonella* in every 2 wk. The result of the study indicated that there was reduction in the bird infection and egg contamination in birds vaccinated with inactivated *Salmonella* Enteritidis (Freitas Neto, et al., 2008).

Mechanism of vaccine protection Salmonella infection in broiler chicken was assayed using either live vaccine (derivative of *Salmonella* Typhimurium serogroup B) or killed vaccine (inactivated oil emulsion bacterin containing *Salmonella* Enteritidis phage types 4, 8, and 13a serogroup D1). Both the birds in live and killed vaccine group were vaccinated at 2 and 4 wk of age, and were orally challenged with $10 \log_{10}$ CFU/ mL of *Salmonella* Enteritidis and euthanized at 6 wk and 7 wk of age respectively. The test on immunological response and *Salmonella* clearance in the birds suggested that in live vaccinated birds there was lower shedding of *Salmonella* in comparison to the control and killed vaccinated birds. (Babu, et al., 2004). Also the cell mediated immune response (Con A and *Salmonella* Enteritidis -flagella) to the pathogen was higher in the

live vaccinated birds than in killed vaccinated birds and control. Killed vaccinated birds had significantly higher humoral response than both the live vaccinated and unvaccinated birds (Babu, et al., 2004).

The route administering vaccine to chicken has also been shown to have effect on the efficacies of the vaccine in offering protection to chicken against *Salmonella* infection. The report of a study where 4 d old birds was vaccinated either orally or intramuscularly prior to been challenged with *Salmonella*. Both group of vaccinated birds had lower shedding rate of the pathogen in their fecal. But a more lasting reduction were observed in the birds vaccinated orally (Barrow, et al., 1990). This is probably because the site of *Salmonella* infection is gastrointestinal (GI) tract, oral vaccinated birds will have higher mucosa secreted antibody than the intramuscularly vaccinated birds.

Other experimental study had indicated that birds could be protected from *Salmonella* infection. This was demonstrated in a study where white leghorn pullets were vaccinated with a phage type 4 *Salmonella* Enteritidis HY-1 at 8 and 12 wk of age. Both vaccinated and control group of birds were either orally challenged with 6 or 3 $\log_{10}$ CFU/ mL of *Salmonella* Enteritidis at 16 wk of age. The liver, spleen, ovary and cecal contents of the both birds in both groups were assayed for *Salmonella* 1 and 2 wk after challenge. And the result of the study suggested that the antibody titer value was in vaccinated birds than in the control birds (Nakamura, et al., 1994b). The concentration of (Davison, et al., 1999) in the cecal dropping of the vaccinated birds was significantly reduced in comparison to control birds (Nakamura, et al., 1994b).

Despite successes observed in the use of inactivated vaccines, some discrepancies in the effectiveness of inactivated vaccines have been reported. Field trial application of inactivated vaccines did not result to reduction in poultry *Salmonella* infection status (Davison, et al., 1999). In this trial 8 out of 11 flocks were given only an initial dose of the vaccine at 16 wk of age. One flock was also given only an initial dose of the vaccine at 20 wk of age. Another flock was also given both an initial and a booster dose at 10 and 14 wk of age respectively. Vaccination was by subcutaneous injection behind leg. The rodents in the poultry house were known to be infected with *Salmonella* and sources of *Salmonella* Enteritidis used for preparation of vaccinate. Organs (liver, spleen, heart, gallbladder, gut, ovary and oviduct) of the birds and environmental (pits for manure, egg belt) samples were collected every month and analyzed for presence of *Salmonella* Enteritidis. And the results of the study showed that all 10 flocks of birds and other flock in the poultry houses were positive *Salmonella* Enteritidis despite being vaccinated with the killed vaccine (Davison, et al., 1999).

Live-attenuated vaccines. These live non-pathogenic vaccines have the ability to induce long-lasting immunity in the host (Curtiss, et al., 1993). Once administered into the host, they are capable of replicating, colonizing and invading the GI tract of the host to elicit immune response (Barrow, et al., 1990; Hassan and Curtiss III, 1997; Mastroeni, et al., 2001). Types of tested live vaccines include; Semi-rough strains (Kwon and Cho, 2011; Silva, et al., 1981), auxotrophic double maker and metabolic drift mutants and genetic gene-deletion mutants. Live vaccines have been effective in both field and

experimental challenges (Atterbury, et al., 2010; Papezova, et al., 2008; Pei, et al., 2014).

In a study in which 20 types of attenuated S*almonella* Typhimurium vaccine strain were tested for their efficacies in protecting young birds from *Salmonella* infection. Birds were vaccinated at 1 and 7 d of age with live attenuated *Salmonella* Typhimurium by direct crop injection. At 14 d of age, 5 birds per treatment group and 10 birds from the control group were orally challenged with 2 x 10^5 CFU/ mL of *Salmonella* Typhimurium and fecal samples were analyzed on 3, 6, 9, 12 and 14 d post-challenge. And at 28 d of age, the birds were euthanized, necropsied then spleen, cecum and cecal contents were assayed for wild strain *Salmonella* Typhimurium. Meanwhile, the fecal content of live birds was also analyzed on 28 d after challenge. And the results of the study showed that live vaccines had ability to persist in and invade the colon of the birds. There was significant reduction in fecal shedding of the virulent strain and its colonization of the cecum in birds inoculated with fast and intermediate growing vaccines (Pei, et al., 2014). The study indicated that the orally administered live attenuated *Salmonella* Typhimurium vaccines are effective in poultry control of *Salmonella* Typhimurium. This might be due to the ability of the vaccines to colonize the GI tract of the birds and offer better protection to the birds.

In another study where birds were vaccinated by oral administration on 1 d of age and were later given booster doses at ages 6 and 16 weeks by either oral or intramuscular vaccinated with live attenuated *Salmonella* Enteritidis mutant strain vaccine. At 24 wk of age, the birds were challenged with 10^7 CFU/ mL of virulent strain of *Salmonella*

Enteritidis by intravenous administration. Further, the humoral, cell mediated and secretory immune status of the birds were IgG and mucosal IgG levels, also the samples were assayed for the presence of the virulent strain of *Salmonella* Enteritidis. And 3 wk post challenge, the chickens were euthanized, necropsied and organs (liver, spleen, ovary and cecum) were analyzed for virulent strain of *Salmonella* Enteritidis. The results of this study indicated that given booster doses orally significantly reduced egg contamination with the pathogen when compared to group vaccinated via intramuscular injection (Nandre, et al., 2014). The study indicates also that live attenuated vaccines administered via oral route will provide better protection against *Salmonella* Enteritidis due to stimulation both cell-mediated and humoral immunity in the hens (Nandre, et al., 2014).

Competitive Exclusion (CE)

This method of pathogenic control is one of the popular and the most acceptable biological strategy for *Salmonella* control in poultry, especially in young chicks. CE is also famously known as Nurmi concept and it involves the collection and culturing of digesta of a pathogen free matured birds and orally administering the digesta into young birds (Nurmi and Rantala, 1973). Young chicks are immunologically not developed and the intestinal microbiota of birds in the early stage of life is also not developed. Chicks within 1 wk of age are very susceptible to *Salmonella* and other enteropathogenic infection. According to a particular study on the vulnerability of birds to *Salmonella* infection, 1 to 10 cells of *Salmonella* may be enough to cause an infection in young birds

(Nurmi, et al., 1992). The effectiveness of few number of pathogen to create an infection in young birds had been attributed to inexistence or inadequate population of other microorganisms in the alimentary canal to inhibit the invading pathogen. The successful reduction of *Salmonella* infection by CE might be due to competition between the pathogen and the microbial constituent of the digesta for nutrient, attachment on mucosa binding site and production of antimicrobial agents (Nurmi, et al., 1992).

Effectiveness of CE in *Salmonella* control was demonstrated in a study when 1-2 d of age chicks were orally given 0.5 mL of saline diluted ingesta (1:10 of ingesta collected from crop and intestinal tract) of healthy adult male chickens. Both the ingesta treated chicks and chicks in control groups were orally challenged either with low dose (10^3) or high dose (10^6) of *Salmonella* infantis, all the chicks were euthanized between 8 to 22 d of age. Result of the study indicated that 23 per cent or 31 per cent of chicks treated with ingesta tested positive to *Salmonella*, while 100 per cent of the chicks in the control groups were *Salmonella* positive (Nurmi and Rantala, 1973).

The effectiveness of *Salmonella* control in young birds was also suggested in another study where a commercially available CE preparation was evaluated. In the study 0.25 mL of the CE product diluted preparation was orally administered to day old chicks and all the birds in the treated and control groups were orally challenged with 10^3 of *Salmonella* Enteritidis. The birds were euthanized on both 5 and 12 d post challenge and ceca, liver, heart and spleen were assayed for *Salmonella* infection. The result of the study indicated that the CE product increased the resistance of the CE treated birds to *Salmonella* infection (Nuotio, et al., 1992). The level of *Salmonella* in the ceca of CE

treated chicks was < 1 $\log_{10}$ CFU/ g of *Salmonella*, whereas in the ceca of birds in the control group the concentration of the pathogen was > 6 $\log_{10}$ CFU/ g.

Also when a continuous flow (CF) culture of adult chicken *Salmonella* free cecal content was administered into day old chicks at a concentration of 8 $\log_{10}$ CFU/ mL of anaerobes. CF culture treated birds that were subsequently challenged at 3 d of age with 4 $\log_{10}$ CFU/ mL of *Salmonella* Typhimurium had lower cecal *Salmonella* infection rate in comparison to control birds (Nisbet, et al., 1993a). The study indicated that the efficacies of the CF cecal culture in reducing cecal *Salmonella* increased with addition of dietary lactose to feed.

Efficacies of CE in *Salmonella* control depends on the *Salmonella* status of the birds that are being treated. Since the concept functions by lowering the opportunity for *Salmonella* survival in the GI tract. Therefore the application of CE might only be suitable for prophylactic purpose alone and this is supported by the result of an in vitro study in which Lactobacilli failed in displacing *Salmonella* that adhered on to epithelial cells (Jin, et al., 1996b).

The limitation of CE may further be compounded by the high *Salmonella* infection rate that chicks are exposed to at the hatcheries (Byrd, et al., 1999; Cox, et al., 1990). The significance of *Salmonella* prevalence in the hatcheries cannot be over emphasized, multiple serovar of the pathogen have been isolated from hatcheries. Therefore, the application of numerous *Salmonella* control strategy is needed to reduce the prevalence of *Salmonell*a on eggs prior to been hatched or else application of CE may not achieve the desired purpose.

Feed and water additives as strategy for *Salmonella* control in poultry

Modulation of poultry feeds is one of the strategies that have been adopted in poultry industry as a strategy for enteropathogenic control in poultry. Since the site of this group of pathogen is in the GI tract and the mode of infection of birds with these pathogens in oral. Feeding birds with these additives from early age will probably have influence on the level of enteric infection in poultry. Several mechanics of feed/ water additives protection in birds had been reported, and some of these groups of additives are discussed in this section.

However, in controlling *Salmonella* infection in poultry, there is no substitute to following all rules of hygiene. Therefore, combination of other interventions with sanitation will effectively reduce the susceptibility of birds to Salmonella infection. And this will subsequently reduce the level of pathogenic contaminants in poultry processing plant.

Antibiotics

Antibiotics are secondary metabolites of some species of microorganisms (mostly molds and bacteria) that have inhibitory effects on wide spectrum of other microorganisms (Jay, et al., 2005). Studies have shown that the inhibitory activities that antibiotics exert on microorganisms may depend on the property of the antibiotics. The inhibitory activities of moenomycin are mainly effective in gram positive bacteria (Huber and Nesemann, 1968), and its bacteriostatic effect on *Staphylococcus aureus* was due to inhibition of cell wall formation (Huber and Nesemann, 1968). An in vitro

evaluation of the effect of SCH27899 on wide range of bacteria indicated the inhibitory effect of the antibiotics was mainly on gram positive bacteria, including the multidrug resistance strains at concentration of ≤ 1.0 µg/ mL (Fuchs, et al., 1999). Meanwhile the antibiotic was ineffective against members of the family of *Enterobacteriaceae* and other non-enteric gram negative bacilli, even when these groups of bacteria were exposed to >8 µg/ mL of the antibiotic (Fuchs, et al., 1999). Antibiotics have been used as growth promoters for decade. Interest in the use of antibiotic as a growth promoter in animal feeds started developing when it was observed that animals fed dried mycelia of *Streptomyces aureofaciens* with residue of chlortetracycline had better growth performance than the animals reared on feeds without the organism (Castanon, 2007).

Modes of action of antibiotics as growth promoter in livestock. The effectiveness of antibiotic as growth promoter was due to its interaction with the microbiota of the alimentary canal of livestock. For instance in the study that investigated the mode of action of selected antibiotic on chicks reported that the size of the intestine and the thickness of gut wall were lower in the birds fed penicillin supplemented feed than in the control birds(Coates, et al., 1955). The study also investigated the effect of feeding antibiotic supplemented feed to germ free chicks, but the growth of the germ free chicks was not affected when fed to feed supplemented with antibiotics. The study suggested and confirmed that antibiotics suppressed the growth of some intestinal microbiota that depressed the growth of livestock by competing with the host for nutrient (Coates, et al., 1955).

Application of antibiotics as a* Salmonella *control strategy in chicken. While feeding of poultry with sub-therapeutic level of antibiotics enhances growth performance in the animal. Supplementing poultry feeds with low level of antibiotic also to increase in the resistance of the birds to pathogenic infection (Evangelisti, et al., 1975; Girard, et al., 1976; Roura, et al., 1992). Both field and experimental studies have revealed the effectiveness of antibiotic application in livestock production in controlling pathogens of health concerns to man.

Report on the effect of the feeding Salinomycin supplemented feed to broiler challenged with *Salmonella* Typhimurium Suggested that the antibiotic did not reduce fecal shedding of *Salmonella* by the birds (Ford, et al., 1981). The report also suggested feeding of feed with salinomycin additive in ratio 80:1 g/ ton of antibiotic to feed may not increase the resistance of *Salmonella* isolate of the antibiotic fed birds to other commonly used antibiotics in the poultry industry (Ford, et al., 1981).

In spite of some of the successes reported on the antibiotic effectiveness in controlling *Salmonella* in food animal. The growing concerns of the public about the emergence of the multi drug resistance bacteria due to the use of antibiotic have imposed pressure on food animal industry to seek other alternatives in controlling human pathogens in farm animals. For instance, investigations on the prevalence of *Salmonella* in poultry and poultry commodities have suggested the presence of antibiotic resistant *Salmonella* in poultry products (Singh, et al., 2010; Yildirim, et al., 2011).

Evidence that zoonotic pathogen might be due to animal farming only was not supported by the survey on the antimicrobial resistant zoonotic pathogen. The result of

the survey revealed that lesser than 4 % of human antimicrobial resistant pathogen of food animal origin (Bywater, 2004). Microorganisms that contaminate human food might be from many sources (Jay, et al., 2005). Similarly antibiotic resistant pathogen in contaminated poultry products might also be from many other sources other than the animal (Phillips, et al., 2004).

Probiotics

Probiotics are group of organisms (mainly bacteria and yeasts) that are believed to offer beneficial effects to intestinal development and functions of both human and animals. However, probiotics have been defined as ‘a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance’ (Fuller, 1989a). Further, other researcher had viewed this definition of probiotics as limiting in the mode of application of probiotics, hence a more elaborate definition of probiotics was suggested in Haveenar and Huis, (1992). A more broaden definition of probiotics quotes as follows: ‘a pro biotic is a mono- or mixed culture of live microorganisms which, applied to animal or man, affect beneficially the host by improving the properties of the indigenous microflora’ (Havenaar and Huis, 1992).

Therefore, for an organism to be described as a probiotic the organism must be able to offer certain benefits to their hosts and these may include: improving the health status of the host organisms and affect the host mucosa lining (Havenaar and Huis, 1992). In poultry industry probiotics are fed to birds for many reasons, one of which is that they offer protection against enteric pathogenic infection in birds (Higgins, et al., 2007;

Higgins, et al., 2010; Line, et al., 1998; Pascual, et al., 1999; Patterson and Burkholder, 2003; Vicente, et al., 2008; Vilà, et al., 2009).

***Application of probiotics as* Salmonella *control strategy*.** In vitro studies of the 12 strains of *Lactobacilli* isolate of avian on 5 strains of *Salmonella* and 3 strains of pathogenic *E. coli* using spot agar tests and well diffusion assay has indicated that *Lactobacillus* spp. are some of the microorganisms that could be used as probiotic to control *Salmonella* infection in chicken. All the *Lactobacillus* strains had inhibitory effect on the pathogens, with *Salmonella* Enteritidis 935/79 and 94/448 and *Salmonella* Pullorum been the most susceptible pathogens to the *Lactobacilli* strains (Jin, et al., 1996a). Similarly, inhibitory effects were also exerted on *Salmonella* Enteritidis and *E. coli* by different chicken *Lactobacilli* isolates (Garriga, et al., 1998; Tsai, et al., 2005). Meanwhile in other in vitro study the results of incubating the *Lactobacilli* and the *Salmonella* with ileal epithelial cells (IEC) was conflicting, however *Lactobacillus acidophilus* I 26 and *Lactobacillus fermentum* I 25 reduced the adherence of *Salmonella* Pullorum and *Salmonella* Typhimurium to the IEC respectively (Jin, et al., 1996b). Variation in the sensitivity of *Salmonella* isolated from poultry and poultry environment was also reported during the in vitro screening of the *Lactobacilli* isolates of cloaca and vaginal of laying hens for probiotic potential (Van Coillie, et al., 2007).

Studies on the effects on probiotics on *Salmonella* infection in poultry have suggested that poultry treated with probiotics are protected from pathogenic infection when day old chicks were orally challenged with 3.8 $\log_{10}$ CFU/ mL of either *Salmonella* Enteritidis or *Salmonella* Typhimurium and were subsequently treated with

oral administration of $\log_{10}$ 5.7 to 6.3 culture of lactic acid bacteria probiotic. Necropsy and *Salmonella* assay of ceca of the birds at different time point (6, 12, 24 h) of treatment indicated that the prevalence and the population of the pathogens that colonized the ceca were significantly reduced within 24 h of the exposure to the pathogens and treatment with the probiotics (Higgins, et al., 2007).

A similar results were also reported in a study where *Lactobacillus salivarius* was administered into the proventriculus of day old chicks and when the probiotic was added to the drinking water and the feed fed to day chicks that were challenged with *Salmonella* Enteritidis, the study suggested that the prevalence of *Salmonella* in the gastrointestinal tract of the birds was reduced to 0 % on the 21 d of sampling (Pascual, et al., 1999). This study also indicated that either proventriculus administration of the probiotics in chicks or supplementing chicks drinking water and feed with probiotics will confer protection against *Salmonella* infection in poultry.

Furthermore, reduction in the prevalence of *Salmonella* was reported in 24 and 72 study where *Salmonella* challenged birds were treated with the application of *Lactobacillus* spp. to drinking water (Vicente, et al., 2008). The effectiveness of some strains of laying hen cloaca and vaginal *Lactobacilli* isolate in protecting *Salmonella* infection in day old chicks have been assayed. Suspension (2×10^8 CFU/ mL) of each of the *Lactobacillus reuteri* (. R-17485), *Lactobacillus reuteri* (R-17753), *Lactobacillus johnsonii* (R-17504) and *Lactobacillus vaginalis* (R-17362) was orally administered into each group of day old birds and birds were subsequently challenged orally challenge with 10^4 CFU of *Salmonella* Enteritidis. The result of the study suggested that there was

variation in the inhibitory effect of the *Lactobacilli* on the pathogen. The population of *Salmonella* that colonized the ceca of the birds treated with *Lactobacillus reuteri* (. R-17485) and *Lactobacillus johnsonii* (R-17504) were significantly reduced in comparison to the control group (Van Coillie, et al., 2007).

The potential use of probiotics as both a prophylactic and therapeutic agent against *Salmonella* infection in young chicks was illustrated in a study with the use of *Lactobacillus acidophilus* that was orally administered to chicks at different time intervals. At 2 d of age chicks was either orally treated with *Lactobacillus acidophilus* or orally challenged with 10^8 CFU of *Salmonella* Typhimurium var *copenhagen*, those birds treated with probiotic were orally challenged with the pathogen on day 4 of the study, and vice versa. Other group of birds in the study was treated with the probiotic before and after been orally challenged with the pathogen and the results of the study indicated that the application of the probiotic as either prophylactic or therapeutic agent reduced the fecal shedding of the pathogen (Watkin and Miller, 1983). This study also suggested that as the fecal shedding of the probiotic increased, the population of the pathogen decreased (Watkin and Miller, 1983).

In addition, the fermentation of moistened poultry feed with probiotic may lower the feed pH and aid in increasing the population of the inoculated probiotic in the poultry feed, hence increase the resistance of poultry to *Salmonella* infection. The concept was demonstrated in a study when poultry fermented liquid feed (FLF) prepared by inoculating each batch of 12.4 kg of poultry starter with slurry prepared from culture of *Lactobacillus plantarium* and 500 g of the feed. The cultured feed batch was incubated

at 30 °C for 2 d and the final concentration of the probiotic was between 10^9 to 10^{10} CFU/g and pH of 4 (Heres, et al., 2003). The report on the study indicated that the fecal shedding of *Salmonella* in the birds that were orally challenged with different concentration of *Salmonella* Enteritidis was significantly reduced in the birds fed with FLF in comparison to the birds fed normal poultry ration (Heres, et al., 2003). Even though there was no difference in the population of *Salmonella* in the ceca. The reduction in the shedding of the pathogen due to feeding with FLF may reduce the level of the pathogen in poultry house, thereby may reduce the incidence of horizontal transmission of *Salmonella* in the flock.

Administration of probiotic singly or in combination on to poultry may increase resistance of chicken to *Salmonella* infection. Application of undefined intestinal ingesta had been successfully used in controlling *Salmonella* in chicken, but the application of defined microbiota of the ingesta resulted to either increase resistance or not having any effect on the resistance of birds to *Salmonella* infection. The study on the use of 3 strains of *Lactobacillus salivarius* and *Streptococcus cristatus* that were isolated from poultry indicated that these organisms have antimicrobial effects on *Salmonella* Typhimurium (Zhang, et al., 2007). Oral administration of culture of these *Lactobacilli* and *Streptococcus* into day old broiler singly and when combined offered high level of protection to the bird against *Salmonella* Typhimurium infection level and prevalence in chicks between 3 d to 10 d of age (Zhang, et al., 2007). The study indicated that administration of the individual 3 strain of *Lactobacillus salivarius* to day old chicks

reduced level of *Salmonella* in the cecal by 2.10, 2.52 and 2.20 $\log_{10}$ and reduced the prevalence of *Salmonella* infection from 84 % to 35 %, 31 % and 35 %.

In addition administration of the mixture of the 3 strains of the *Lactobacillus salivarius* and *Streptococcus cristatus* to day old birds in 2 trials reduced the cecal concentration of Salmonella Typhimurium by 2.2 and 4 $\log_{10}$ and the prevalence of the pathogen from 90 % to 65 % and 88 % to 31 % in each of the trial (Zhang, et al., 2007). But the study also suggested that the probiotics were not effective in controlling *Salmonella* Enteritidis and *Salmonella* Kentucky infection in the chicks (Zhang, et al., 2007).

Results of feeding of young chicks after hatch as described in the Nurmi Concept suggested that the practices will protect poultry from enteropathogenic infection such as *Salmonella*. Similarly, application of probiotic in drinking water and feed increased the resistance of poultry to *Salmonella* infection. However, in a study that tested the ability of *Lactobacillus* to reduce *Salmonella* infection in poultry by administering *Lactobacillus* on to newly hatched poultry through drinking water indicated that the probiotic did not protect the chicken form *Salmonella* infection (Adler and DaMassa, 1980).

Other studies have also indicated that microorganisms of other genus could also be used as probiotics in poultry industry. In a particular study groups of chicks were orally challenged with *Salmonella* Enteritidis at different days of age (3, 7, 14 d), fed on feed supplemented with of *Bacillus cereus var. toyoi* at ratio 1:1000 g/ kg of probiotic to feed. And the result of the study indicated that at 42 d of age the prevalence of *Salmonella* in

the challenged and probiotic treated birds was reduced from 42 % to 0 % (Vilà, et al., 2009), this study also revealed that probiotic might be capable of protecting chicken against *Salmonella* infection at any rearing stage of the broiler production. However the results on the timing of the administration of probiotics in poultry may be inconsistent, since studies results have demonstrated that probiotic application may be ineffective if the birds are already infected with pathogen (Higgins, et al., 2010).

Different probiotics organisms may have different inhibitory effects and mode of protecting *Salmonella* colonization in the GI tract of poultry. Hence the timing of poultry treatment with probiotics may have impact on its efficacies of offering protection against pathogenic colonization of the intestine. Therefore the protection offered to birds by probiotics against *Salmonella* infection may only be achieved if the birds being treated have not been previously exposed to the pathogen (Cox, et al., 1990).

Ability of yeast to inhibit the growth of pathogenic organisms in the intestine of poultry was illustrated in studies that supplemented avian feed with yeast. *Saccharomyces boulardii* was added to poultry feed in either ratio 1:1 g/kg, or 100: 1 g/ kg of the yeast to feed. The feed was fed to the birds in the challenge group while the control group was standard starter feed and was fed to the birds in the control groups. At age of 4 d chicks were orally challenged with 3.3×10^8 CFU/ mL of *Salmonella* Typhimurium and 6.5×10^8 CFU/ mL of *Campylobacter jejuni*. And the results of the microbiological analysis of the birds indicated that the *Salmonella* colonization of the ceca was significantly reduced by feeding birds with *Saccharomyces boulardii* for 23 d. The mean populations of the pathogen in the ceca were 1.64 $\log_{10}$ CFU/ g, 0.35 $\log_{10}$

CFU/ g and 0.15$\log_{10}$ CFU/ g in the control feed, 1:1 g/kg, and 100: 1 g/ kg of the yeast to feed respectively (Line, et al., 1998). Also the prevalence of *Salmonella* was significantly higher in the control (60 %) compared to 15 % and lower in the *Saccharomyces boulardii* fed birds (Line, et al., 1998), this study indicated that feeding poultry with *Saccharomyces boulardii* could reduce *Salmonella* infection in poultry.

However, the effect of feeding poultry with feed supplemented with *Saccharomyces boulardii* in ratio 50: 1 mg/ kg of yeast to feed on the prevalence and the level of *Salmonella* infection in poultry was also assessed. In the study day old chicks were fed with the yeast supplemented ration for 15 d prior to been orally challenged with 6.3 $\log_{10}$ CFU/ mL of *Salmonella* Enteritidis. And the prevalence and level of the poultry infection with the pathogen were analyzed on the 35 d of the study. The result of the study indicated that the level of the pathogen in cloaca, breast and the prevalence of the pathogen in the neck were significantly reduced in the yeast fed pathogen challenged birds, but the prevalence and the level of the pathogen in the ceca digesta were not reduced by the yeast supplement (Mountzouris, et al., 2015).

There was disparity in the effectiveness of yeast application as probiotic in both studies, meanwhile this might be due to the differences in the concentration of the yeast applied in both studies. Reports have suggested that the efficacies of probiotics intervention strategies in controlling pathogen infection in poultry may depend on the concentration of the probiotic administered. And the concentration effect of yeast supplement on *Salmonella* infection in poultry was demonstration in Line, et al. (1998), the report indicated that the prevalence and level of the pathogen in the poultry fed with

ratio 1:1 g/kg and 100: 1 g/ kg of the yeast to feed not equal, therefore the level and prevalence of *Salmonella* detected in the ceca digesta of the yeast fed poultry reported in Mountzouris, et al. (2015) may be due to lower concentration of the yeast supplement applied to the feed.

Prebiotics

Generally, prebiotics are described as non-digestible food/ feed ingredients that beneficially affect the host by selectively stimulating the growth and or activity of one or a limited number of bacterial species that are residents of the colon and thus attempt to improve host health (Glenn and Roberfroid, 1995). This implies that prebiotic will not be metabolically utilized by the animal or human feeding on it. However, the benefit derived by the host for feeding on prebiotics will manifest in the increased population or the activities of the host normal microbiota that can metabolize prebiotics. Most common member of the GI tact microbiota that is targeted for increase growth and activities are the member of genera *Lactobacilli* and *Bifidobacteria* (Manning and Gibson, 2004). Depending on the host GI tract normal microbiota, most ingredients classified as prebiotics belong to carbohydrate class of food, which could be monosaccharide, disaccharide, oligosaccharide or polysaccharide (Manning and Gibson, 2004). According to Manning and Gibson, (2004) for food/feed material to be qualify as prebiotic, it must possess the characteristics that include; i. The ingredient must not be digested or absorbed in the stomach/ proventriculus or in the small intestine. ii. The ingredient must be selective for beneficial microbiota such as member of *Lactobacilli*

and *Bifidobacteria* that resides in the colon. iii. The product of the fermentation of the ingredient must have beneficial effects on the host.

In poultry, bacteria members of *Lactobacillus* and *Bifidobacterium* species are some of the bacteria that have been shown to offer protect against *Salmonella* and other enteropathogens intestinal colonization (Carter, et al., 2009; Gusils, et al., 1999a; Gusils, et al., 1999b; Mishra and Lambert, 1996; Zhang, et al., 2007). *Lactobacillus* species is one of the dominant organisms in the ceca of poultry (Barnes, et al., 1972; Mead and Adams, 1975). Hence addition of prebiotics may be one of the strategies that actually protect chicken from *Salmonella* infection, since the ingredient will promote the growth and activities of *Lactobacilli* and *Bifidobacteria* species (Grizard and Barthomeuf, 1999; Ishihara, et al., 2000) in the large intestine. Studies have suggested that efficacies of *Lactobacillus* in reducing level of enteric pathogen in poultry may depend on its population (Lee, et al., 2000). Therefore, prebiotic application may directly or indirectly protect poultry from pathogenic infection.

Results of experimental studies had suggested that feeding poultry with diet containing prebiotic may reduce the susceptibility of the birds to *Salmonella* infection (Eeckhaut, et al., 2008; Ishihara, et al., 2000; Spring, et al., 2000b). Studies on feeding of birds with 4000 ppm of dietary mannanoligosaccharide extracted from yeast have indicated that there was reduction in the prevalence of *Salmonella* in the prebiotic treated birds. In this study 3 d old birds that were previously treated with *Salmonella* free digesta of known microbiota were orally challenged with 10^4 CFU/ mL of one of the *Salmonella* serovar (*Salmonella* Typhimurium 29 E, *Salmonella* Dublin, *Salmonell*a

Typhimurium 27 A). And were assayed for *Salmonella*, pH, and cecal lactic acid bacteria. The results of the study suggested that there was a significant reduction in the cecal level of *Salmonella* Typhimurium 29 E in the prebiotic fed birds (Spring, et al., 2000b). In birds challenged with *Salmonella* Dublin, the prevalence of pathogen was significantly reduced in the prebiotic fed birds (56 %) in comparison to the control birds (90 %). Meanwhile the coliform, *Lactobacillus*, *Enterococcus*, anaerobes, lactate, volatile fatty acid and pH of ceca were not significantly modified by the prebiotic (Spring, et al., 2000b). Addition of other mannose containing ingredient such as palm kernel meal in the poultry diet may also protect poultry from *Salmonella* infection (Allen, et al., 1997).

Furthermore, feeding of diet containing arabinoxylooligosaccharides (AXOS), a hydrolyzed product of arabinoxylan from wheat bran at concentration levels of 0.4 % and 0.2 % had been shown to reduce the prevalence and concentration of *Salmonella* infection in birds (Eeckhaut, et al., 2008). In this study 224 birds were divided into 4 groups and were fed with either no AXOS or fed AXOS but at different concentration prior to been orally challenged with 2.5 x 10^9 CFU/ mL of *Salmonella* Enteritidis at 14 d of age. The prevalence of *Salmonella* in the cloaca swabs of birds fed 0.4 % AXOS was significantly lower on 1, 3, and 11 d, also the level of the pathogen in the cecal content was significantly reduced. A similar reduction in the prevalence of cloaca swab *Salmonella* was also observed in the birds fed 0.2 % AXOS on 3 and 11, but in the birds fed with 0.2 % of second category of AXOS, significant reduction in the prevalence of cloaca was *Salmonella* was observed only on 11 d post challenge with the pathogen

(Eeckhaut, et al., 2008). The prevalence of *Salmonella* in the spleen was significantly reduced in all the groups fed AXOS.

Supplementing feed with partially hydrolyzed guar gum (PHGG) may also offer certain level of protection to poultry and eggs against *Salmonella* infection. This was demonstrated in a study where 9 wk of age pullets was fed with feed that contained different concentration of PHGG for a week prior to been orally challenged with 3.2 x 10^6 CFU/ mL of *Salmonella* Enteritidis. The result of the analysis of birds from 1 to 21 d post challenge with the pathogen suggested that prevalence of *Salmonella* in the organs of birds was significantly reduced to 5.6 % when the feed was supplemented with 0.025 % of PHGG (Ishihara, et al., 2000). Whereas in the control fed birds the prevalence of *Salmonella* was 26.7 %. Similarly, the prevalence of pathogen was significantly reduced to 16.7 % and 12.5 % in laying hens fed 0.025 % of PHGG and laid eggs respectively, unlike 63.3 % and 34.5 % prevalence of the pathogen in the hens fed control feed and eggs respectively (Ishihara, et al., 2000).

Lactose, a disaccharide comprising a molecule of glucose and galactose, has also been shown to play a role of prebiotic in poultry. About 50 % of dietary lactose in poultry diet was not digested prior to reaching the large intestine where it was fermented. Experimental studies have revealed the dietary importance lactose in controlling *Salmonella* infection in young poultry. Day old chicks that were supplied with diet containing 10 % of lactose for 13 and 18 d prior to being challenged with 10^8 CFU/ mL of *Salmonella* Enteritidis had lower prevalence of *Salmonella* in 24 h after been challenged.(Tellez, et al., 1993). Also the result of the analyses of the ceca pH,

concentration of acetic acid, propionic acid, butyric acid and lactic acid suggested that in lactose fed birds, the pH decreased and the concentration of all the acids increased significantly (Tellez, et al., 1993). However, the result of the effect of providing drinking water with lactose concentration of 2.5 % to 7 wk old broiler for either 5, 11 or 15 d post challenge with 10^8 CFU/ mL of *Salmonella* Enteritidis and during feed withdrawal period (18, 24 and 12 h) did not reduce the prevalence of the pathogen in the crop and ceca (Barnhart, et al., 1999).

Feeding of poultry with diet containing fructooligosaccharide (FOS) has also been shown to reduce level of *Salmonella* colonization of the ceca. In a study evaluating the efficacies of using 0.1 % of FOS ingredient in poultry diet, the result of the study indicated that the level of *Salmonella* Enteritidis in the ceca of the birds wa significantly reduced on 1 and 7 d post challenge with the pathogen (Fukata, et al., 1999). Meanwhile the concentration of the total microbes, *Bacteroides, Bifidobacterium, Lactobacillus* and *E. coli* in the ceca were not significantly affected by the prebiotic (Fukata, et al., 1999).

Meanwhile Bailey et al, (1991) report on the studies of effect of FOS on birds orally challenged with *Salmonella* Enteritidis indicated that providing birds with drinking water containing 2 % of FOS was ineffective in protecting the birds against *Salmonella* infection. Also addition of either 0.375 % or 0.75 % of FOS did not significantly protect the birds from *Salmonella* infection. But administration of CE and feeding with FOS significantly reduced the prevalence and concentration of *Salmonella* in birds. The study also suggested that birds stressed by feed and water withdrawal were protected from *Salmonella* infection when fed 0.75 % of FOS (Bailey, et al., 1991). Also provision of

drinking water containing 2.5 % of mannose to birds orally challenged with 7.2 x 10^8 CFU/ mL of *Salmonella* Typhimurium significantly reduced prevalence of *Salmonella* in birds when compared to the control birds (Oyofo, et al., 1989).

However, despite the reports on the reduction of *Salmonella* infection in birds fed with prebiotics, there are reports that indicated the ineffectiveness of some prebiotics in protecting birds against *Salmonella* infection (Ribeiro, et al., 2007). Also some inconsistences in the efficacies of feeding prebiotics to birds have been reported, for instance in the case of lactose, while feeding lactose to bird successfully supported the inhibition of growth and survival *Salmonella* in the GI tract of some birds (Tellez, et al., 1993). The administration of lactose to broiler through drinking water was ineffective in controlling *Salmonella* infection in broiler chicken (Barnhart, et al., 1999). Also inconsistence in birds fed FOS has also been reported.

Synbiotics

Synbiotic*s* are synergistic combinations of prebiotics and probiotics (Collins and Gibson, 1999; Schrezenmeir and de Vrese, 2001). They possess immunostimulatory properties that aid in maintaining the epithelial integrity of poultry intestines. In this era of antibiotic-free poultry production due to increasing antibiotic resistance concern, synbiotics have gained much popularity from their ability to stimulate and establish proper intestinal microbiota balance via competitive exclusion of pathogenic bacteria preventing pathogen-related disorders in the birds. Synbiotics have an added advantage of promotor and early maturation of beneficial bacterial growth in young birds which are

more susceptible to pathogenic infection. And this enhances early intestinal colonization by beneficial bacteria which is vital for nutrient digestion, absorption and thus, growth of the chicks. Inclusion of lactitol and *Lactobacilli* (Collins and Gibson, 1999), FOS and *Bidobacteria,* Bacterial Culture and Dietary Lactose (Nisbet, et al., 1993b) have been shown to exert anti-microbial effects on *Salmonella.*

Treating of young birds with a synbiotic which comprised bacterial culture and dietary lactose against when the birds were orally challenged with *Salmonella* Typhimurium challenge revealed that cecal level of *Salmonella* was reduced in all chicks administered the lactose + CF culture (Nisbet, et al., 1993b).

Despite successes reported on the effect of synbiotics in *Salmonella* control. Some discrepancies had also been reported. For example, failure of a synbiotic added to feed fed to laying hens and broilers to prevent *Salmonella* infection in the birds. Both bird types were orally challenged with *Salmonella* Enteritidis at 1 d of age. The birds had unrestricted access to the feed supplemented with the synbiotic. On days 7, 14 and 21 after been challenged with the pathogen, cloacal swabs and cecal content of the laying hens and the broiler were assayed for Salmonella. In addition, cecal contents of broiler were also sampled on days 2 and 5 post challenge. The results of the study suggested that the synbiotic did not offer protection to the birds against *Salmonella* infection (Sayuri Murate, et al., 2014).

Fatty acids

The lethal effect of short fatty acid on some food pathogens has been of research interest over the past decades. Generally, organic acids are effective in deactivating bacteria, this is because they have poor dissociating property especially in reduced pH matrices. Organic acids dissociate once they diffuse into microorganism cell, and inhibit the cellular functions of the organism (Jay, et al., 2005). However, the efficacy of organic acid in pathogenic control varies, and the variation in the effect of microorganism control is also applicable to short chain fatty acid.

The result of in vitro study on the effect of short chain fatty acid on pathogenic *E. coli* and *Salmonella* spp. had suggested that this group of fatty acid exerted different degree of inhibition to microorganisms. Exposure of the pathogens to 0.5 mol/ L of propionic and formic acid indicated that both acids killed 90 % of the pathogens (Cherrington, et al., 1991). However the 90 % lethality effect of propionic acid was achieved in 1 h, whereas the inhibitory effect of formic acid was not achieved until between 3.7 and 11.8 h of exposure to the pathogens (Cherrington, et al., 1991).

Other mechanisms of short chain fatty acid may be due to their ability to have effect on gene expression of pathogen. For example, butyrate has been shown to down regulate a total of 49 and 90 genes in *Salmonella* Typhimurium and *Salmonella* Enteritidis respectively (Gantois, et al., 2006). Out of all the downregulated genes, 23 and 24 genes were involved in the cell invasion associated with *Salmonella* Pathogenicity Island 1 (SPI1) of *Salmonella* Typhimurium and *Salmonella* Enteritidis respectively (Gantois, et al., 2006). Meanwhile an in vivo study on rodent also suggested that the butyrate and

propionate in the GI tract significantly reduced the invasion level of *Salmonella* Typhimurium. By downregulating the expression of genes that encoded for this virulence factor, but the exposure to acetate actually restored and upregulate the expression of the genes.(Lawhon, et al., 2002).

Due to the inhibitory effect of volatile fatty acid on *Salmonella*, poultry industry has developed a strategy of increasing the concentration of volatile fatty acid in birds to serve as one of the strategy of controlling *Salmonella* infection. The volatile fatty acid concentration in the ceca of birds has been increased by feeding birds with probiotics (Meimandipour, et al., 2010), prebiotic, synbiotics and salt of short chain fatty acid. For example treating poultry with pathogen free cecal culture and feeding dietary lactose or adding lactose to drinking water of poultry had resulted to increase the cecal concentration of undissociated propionic, acetic and butyric fatty acid (Corrier, et al., 1990). Also the prevalence and level of *Salmonella* infection were significantly reduced in the lactose treated birds. Furthermore, addition of sodium butyrate of 0.92 g either protected with vegetable oil or unprotected to 1 kg of broiler feed was shown to reduce fecal shedding of *Salmonella* (Fernández-Rubio, et al., 2009). And the prevalence of the pathogen in the crop and ceca of the birds fed with feed containing the butyrate salt was significantly reduced (Fernández-Rubio, et al., 2009).

Report has also suggested that feeding birds with feed acidified with sodium salt of either formic or propionic acid that contain 1 % of either free formic or propionic acid protected birds from *Salmonella* infection (McHan and Shotts, 1992). In this study, day old birds were fed with feed containing either 1 % propionic acid or formic throughout

the study. The birds were orally challenged on 4 d with 10^6 CFU/ mL of *Salmonella* Typhimurium and the level of *Salmonella* concentration in the ceca of the birds were determined on days 7, 14 and 21 of the study. The cecal *Salmonella* level was reduced by log 1.4 on 7 d, log 2.56 and 3.09 on 14 d in the short chain fatty acid fed birds. And on 21 d the cecal concentration of the pathogen in either the formic or propionic fed birds was reduced by log 3.6 (McHan and Shotts, 1992).

Study on the bactericidal effect of some dietary short chain fatty acid had suggested that the dietary intake of SCFA may not alter the pH of crop and gizzard of birds and concentration of the acid may be adequate to inactivate *Salmonella* (Thompson and Hinton, 1997). In the study fed 1 yr of age laying hens was diet containing formic acid and propionic acid in ratio 4.6:1 g/ kg and 1.4:1 g/kg acid to feed respectively for 1 wk. The crop and gizzard of the hens were analyzed for pH and concentration of undissociated propionic, formic and lactic acid. The result indicated that the pH of the organs was not affected by the feed, but the concentration of both propionic and formic acid increased in both the crop and gizzard (Thompson and Hinton, 1997). While the concentration of lactic acid decreased, which means that lactic acid bacteria might have been adversely affected by the dietary SCFA (Thompson and Hinton, 1997). Furthermore, simulation of the pH and concentration of the undissociated propionic, formic and lactic acid in the crop was shown to have inhibitory effect on *Salmonella* (Thompson and Hinton, 1997).

While several studies have shown an increase in the resistance of birds fed dietary SCFA to *Salmonella* infection. Result of some studies has shown that dietary intake of

SCFA may have effects on intestinal microbiota such *Enterococcus* and *Lactobacillus* (Van der Wielen, et al., 2000). The study indicated that higher cecal concentration of *Enterobacteriaceae, Enterococcus* and *Lactobacilli* before 3 d of age. But the cecal concentration of *Enterobacteriaceae* and *Enterococcus* started decreasing while the concentration of undissociated SCFA increased in GI tract of birds up to 15 d of age (Van der Wielen, et al., 2000). And when 5 members of *Enterobacteriaceae*, 4 strains of *Enterococci* and 1 *Lactobacillus* of cecal isolates were cultured in cultural media with inclusion level of undissociated butyrate, acetate and propionate similar to concentration in cecal of birds at different age. The result of this in vitro study suggested that as the concentration of the undissociated SCFA increased with the age of birds, the sensitivity of the 5 members of *Enterobacteriaceae* to this SCFA increased (Van der Wielen, et al., 2000). Also the 4 strains of *Enterococci* were inhibited with increasing level of undissociated SCFA, but the growth Lactobacillus isolate was not affected by the acids (Van der Wielen, et al., 2000).

In the market SCFA administered onto poultry are available in either powdery or encapsulated form. Butyric acid is one of the SCFA feed additives that have reportedly reduced susceptibility of birds to *Salmonella* infection in bird (Van Immerseel, et al., 2004b). Result of the efficacies of powder, encapsulated and combination of both forms of butyric acid feed additive on young laying hens that were orally challenged with *Salmonella* Enteritidis suggested that the encapsulated butyric acid was more effective in controlling *Salmonella* infection in birds (Van Immerseel, et al., 2005). Also the prevalence of *Salmonella* in the ceca and internal organs of birds fed encapsulated

butyric acid was significantly lower than in the control and other forms of butyric acid fed groups. Also fecal shedding of Salmonella was reduced in broilers that were experimentally challenged birds with the pathogen when fed on feed containing encapsulated butyric acid (Van Immerseel, et al., 2005).

Acidification of drinking water with SCFA such as formic and lactic acid given to broiler chickens during feed withdrawal had been shown to reduce the level and prevalence of *Salmonella* in crop of broilers. And this was demonstrated in both experimental and field settings. In an experimental study broiler chicken were orally challenged at both 35 and 41 d of age with 10^8 CFU/ mL of *Salmonella* Enteritidis. On 42 d of age the birds were provided with the acidified water 8 h (feed withdrawal period) prior to been euthanized. This drinking water was acidified with 0.5 % of either acetic, formic or lactic acid. And the result of crop analysis for *Salmonella* indicated that there was significant reduction in the level of *Salmonella* compared to the control birds (Byrd, et al., 2001). Furthermore, the prevalence of *Salmonella* in the crop of the birds that drank water acidified with either formic or lactic acids was significantly reduced.

Meanwhile in the commercial broiler grow out houses 0.44 % of lactic acid was used to acidify drinking water provided to the treatment birds during feed withdrawal period. And the prevalence *of Salmonella* in the crop was significantly reduced in the birds provided with acidified water at post feed withdrawal assay (Byrd, et al., 2001). This study indicated that reduction in the level of *Salmonella* in birds at the preharvest stage reduced the incidence of the carcass contamination during harvesting. For example, the reduction in the prevalence of *Salmonella* in the crop of the treated bird positively

correlated to the reduction in the incidence of carcass contamination at the pre chilling unit operation stage of chicken processing (Byrd, et al., 2001).

Apart from application of SCFA as poultry feeds additive, the feeding of feed containing medium chain fatty acid (MCFA) to poultry may also increase resistance of birds to *Salmonella* infection. Report on in vitro and in vivo study on the effect of caproic, capric and caprylic on *Salmonella* invasion have suggested the ability of some MCFA in increasing host resistance to the pathogen (Van Immerseel, et al., 2004a). In the in vivo study 1 d old birds were fed feed containing caproic acid in ratio 3:1 g/kg of the acid to feed. The birds were challenged with 3 x 10^3 CFU/ mL of *Salmonella* Enteritidis on 5 d, then cloaca swabs and organs (cecum, liver and spleen) were sampled on 6 and 8 d respectively. In birds fed caproic containing feed, the prevalence of the pathogen in the cloaca and concentration in the ceca and liver was significantly reduced in comparison to the bird fed control feed (Van Immerseel, et al., 2004a).

However, despite the efficacies of propionic on deactivation of several serovars of *Salmonella* in in vitro study (Cherrington, et al., 1991). Feeding poultry with diet containing 30 µmol of dietary propionic acid per g of feed increased the concentration of the acid in the crop, however dietary propionic acid did not reduce pH and the prevalence of *Salmonella* in the crop and ceca of the birds (Hume, et al., 1993).

Similarly feeding of feed supplemented with either acetic, formic or propionic acid of concentration levels of 0.24 %, 0.22 % or 0.27 % respectively had failed to reduce intestinal and systemic *Salmonella* infection in young broilers (Van Immerseel, et al., 2004b). Study had also shown that mixture of different SCFA in the intestine might

increase expression of virulence factor *Salmonella* Typhimurium (Lawhon, et al., 2002). Therefore, search for feed ingredients that will metabolize to a desirable SCFA may be considered when mixing feeds.

Experimental chlorate product

Chlorate is an anion with a chemical formula of ClO_3-, and can combine with a cation, mostly a metal to form salt. Chlorate is a bye product formed during photodecomposition of ClO_2, Cl_2 and ClO_4 in water (Siddiqui, 1996). During anaerobic metabolic activities some microorganisms are capable of using chlorate as a terminal electron acceptor (Logan, 1998) and reduced the anion to simpler compounds (Bruce, et al., 1999; Bryan and Rohlich, 1954; Malmqvist, et al., 1991). Chloride is one of the products formed when chlorate is reduced (Bruce, et al., 1999; Rikken, et al., 1996), formation of chlorite during metabolic reduction of chlorate compound has also been reported (Quastel, et al., 1925; Roldan, et al., 1994). However metabolic utilization of chlorate by some bacteria may be reduced in the presence of other substrates such as nitrate (De Groot and Stouthamer, 1969; Van Ginkel, et al., 1995).

Several study results have demonstrated the effectiveness of administering ECP to water or feed in controlling *Salmonella* infection in poultry. When 6 wk of age broiler feeds were supplemented with 0.5 %, 1 %, 5 %, 10 %, 18.5 % or water with ECP for 1 wk after been orally challenged with *Salmonella* Typhimurium (0.7 to 1.8 x 10^9 CFU/ mL). The birds were deprived access to feed 8 to 10 h prior to been euthanized, the results of the *Salmonella* test on crop and ceca indicated that Prevalence of *Salmonella*

in the crop and ceca was significantly reduced in the birds fed with feed supplemented with ECP from concentration of 5 % and up (Byrd, et al., 2008). Also the concentration of *Salmonella* in the crop was reduced in the broiler groups fed 10 % ECP and ECP water. However the pathogen in the ceca was significantly reduced in birds fed feed containing ECP of 5 % and higher (Byrd, et al., 2008).

Six weeks of age broilers were provider either drinking water, 0.5 x, 1 x, or 2 x ECP and all the birds were orally challenged with 10^8 CFU/ mL of *Salmonella* Typhimurium (41 d) 1 d prior to the end of the study. And 10 h prior to euthanasia, all the birds were subjected to 10 h feed withdrawal. Crop and ceca were sampled for *Salmonella*, and the result suggested that prevalence and concentration of the pathogen were significantly reduced in the crop of the birds in all the ECP treated groups (Byrd, et al., 2003).

Environmental stimuli in intensive poultry farming system

Lighting system in poultry industry

Intensive poultry farming entails provision of all the factors that is required for the general well-being of poultry since the motor activities of birds may be affected (Blatchford, et al., 2012; Blatchford, et al., 2009), unlike in the extensive poultry farming where the birds are free to fend for themselves. In the modern day poultry production, which is predominantly intensive poultry farming system, one of the factors of importance is the lighting of the poultry houses. Energy consumption is expensive and energy needs to be judiciously utilized to minimize cost and ensure effective poultry

productivity (Appleby, et al., 1992; Buyse, et al., 1996; Rahimi, et al., 2005; Scheideler, 1990). Therefore, any characteristic of light energy that reduces cost and maximize poultry productivities should be harnessed. Some of the characteristics of lighting system on poultry farm that have been manipulated for either cost reduction or improved poultry productivity performance includes the intensity of light (Deaton, et al., 1981; Hughes and Duncan, 1972; Newberry, et al., 1988), photoperiod (Classen, et al., 1991; Simmons, 1982; Wilson, et al., 1984), source of light (Boshouwers and Nicaise, 1993) and wavelength of light (Prayitno, et al., 1994).

Impacts of lighting parameters on poultry health

Lighting protocols have been linked to poultry health. Studies on chickens between 0 to 3 wk of age indicated that mortality of the chicks in the poultry house lit with continuous light intensity of 75 lux was significantly lower than in the house with continuous light intensity of 5 lux (Deaton, et al., 1981). Report of a similar study also implied that birds reared under 180 lux had significantly lower mortality rates than those reared under 6 lux (Newberry, et al., 1988). The incidence of leg disorders were higher in birds reared under dim light (Blatchford, et al., 2012; Newberry, et al., 1988). Other studies suggested that lighting intensities did not play any significant role on the health of poultry. Immune responses, diameter of the eyes and the gait score of 1 to 6 wk old chicks reared under 5 lux, 50 lux and 200 lux were not significantly different (Blatchford, et al., 2009). These results are in contrast to reports that stated that the

incidence of eye abnormalities was high among birds reared under low intensities (Blatchford, et al., 2012; Buyse, et al., 1996).

Reports on investigation of photoperiod have revealed that the extent of increase in photophase may have impact on health of poultry and that the gradual increase of photoperiod over time resulted to a lower incidence of skeletal disease, sudden death syndrome and mortality than in the birds reared under near-continuous lighting (Classen, et al., 1991). Also the incidence of twisted leg was lower in the flock reared under intermittent lighting system (Simmons, 1982; Wilson, et al., 1984). Birds reared under continuous lighting are also more predisposed to eye abnormalities such as glaucoma and hyperopia (Lauber, 1987; Lauber, 1991; Li, et al., 1995). Result of studies in which birds were reared under continuous lighting had also suggested that birds reared under such rearing condition may experience physiological stress than those reared under 12L:12D (Freeman, et al., 1981). The immune response of the birds reared under continuous lighting was lower and delayed, unlike those reared under 12L:12D (Kirby and Froman, 1991).

In summary, the health status of birds reared under long hours of lighting may be compromised, which may reduce the resistance to infectious agents.

Roles of lighting parameters on poultry performance

Some studies have indicated that lighting program can have direct or indirect effect on the poultry performance indices such as feed intake, muscle development, body weight, feed conversion and yield (Lien, et al., 2007; Ohtani and Leeson, 2000; Rahimi,

et al., 2005; Renden, et al., 1991). Renden, et al. (1991) revealed that the performance of birds raised under different photoperiod differs, with higher performance yield observed in birds reared under long photoperiods. Other reports have suggested that performance of birds reared under intermittent lighting system was superior to birds reared under continuous lighting. Especially when feed intake in correlation with body weight was measured (Ohtani and Leeson, 2000; Simmons, 1982).

Studies have also indicated that light intensity did not have impact on body weight and feed consumption of birds (Blatchford, et al., 2009; Deep, et al., 2010; Newberry, et al., 1988). However, the carcass, thigh and drum yield can be affected by light intensity. Increased yields were observed as light intensity decreased (Deep, et al., 2010). Report of the study on the extent of the reduced light intensity that promoted productivity suggested that with light intensity that ranged from 0.1 to 10 lux resulted in performance and breast yield directly correlated to light intensity (Deep, et al., 2013).

The light sources did not affect the productivity performance of birds in a field trial where the performance of birds reared under incandescent lighting was compared to the performance of the birds reared under fluorescent lighting (Denbow, et al., 1990; Scheideler, 1990).

Effect of lighting system on the activities of chickens

The activity of birds could be affected by the property of the lights under which they are reared. Reports on lighting intensity, photoperiod, wavelength and light source have indicated that at least one of the lighting parameters have effect on birds movement

(Blatchford, et al., 2009; Boshouwers and Nicaise, 1993; Buyse, et al., 1996; Lewis and Morris, 2000; Newberry, et al., 1988; Prayitno, et al., 1994; Simmons, 1982). Blatchford et al. (2009) compared the activities of chicken broilers reared under 5 lux, 50 lux and 200 lux from 1 to 6 week of age. The study revealed that birds reared under 5 lux were less active than those reared under the other higher light intensity categories. Similarly, Newberry et al. (1988) monitored the frequency of standing, walking and total motor activities of birds reared in rooms lit with 180 lux and 6 lux and found that lesser activities were observed in the birds reared under 6 lux.

Studies on the effect of photoperiod on activities of bird implied that birds reared in a continuous lighting condition were less active than those reared under intermittent lighting (Simmons, 1982).

The physical activities of birds may also be affected by the lighting. Birds showed higher physical activities when reared in rooms lit with fluorescent light than when the rooms were lit with incandescent light bulb when the light intensity was higher than 5 lux (Boshouwers and Nicaise, 1993). Meanwhile, the behavior of birds such as pecks, pecks and pull were not influenced by the source of lighting (Denbow, et al., 1990).

Lighting system and transmission of **Salmonella** *in chickens*

Different features of lighting systems have been shown to have impact on health, feeding behavior, and activities of birds (Blatchford, et al., 2009; Boshouwers and Nicaise, 1993; Hughes and Duncan, 1972; Kirby and Froman, 1991; Prayitno, et al., 1997; Simmons, 1982; Xie, et al., 2008). Considering the mechanisms of *Salmonella*

infection in live birds, the choice of lighting system for rearing chicken may directly or indirectly increase poultry susceptibility to *Salmonella* infection (Volkova, et al., 2010). Motor activities of birds such as walking, wing flapping, preening, litter pecking, feather pecking, aggressive behavior and sunbathing may be influenced by the lighting system. And all these motor related activities will increase dust generation in the poultry pens (Al-Homidan, 2004; Calvet, et al., 2009; Ellen, et al., 2000). Dust particles have been shown to be one of the likely vehicles for *Salmonella* transmission in poultry farms (Harbaugh, et al., 2006; Jay, et al., 2005; Marin, et al., 2011; Mitchell, et al., 2002; Mitchell, et al., 2004). Increased activities of birds could lead to dust generation from feed, litter, dried fecal matter and feathers with all these materials are potential sources of *Salmonella*. Settling of dust containing viable *Salmonella* cells on contact surfaces, feed and drinker can promote horizontal transmission of the infectious agents to uninfected poultry in the flock. Therefore, the lighting management should be designed in such a way that the activities of the birds will not result to excessive generation of dust which might transmit *Salmonella* throughout the poultry farm.

In a research that investigated the relationship between lighting program, motor activities of birds, and the concentration of dust generated indicated that there was a linear relationship between the length of photophase, motor activities and dust generation. Birds raised in photophase had 4 times the amount of dust generated versus the dust generated during the scotophase (Calvet, et al., 2009). There was also a difference in the concentration of the inseparable dust generated under different lighting regimens but with same light source and intensity. This study indicated that more dust

was generated in the birds reared under intermittent lighting program (3L:1D) when compared to the amount of the dust in the pen lit with near continuous lighting system of 23L:1D (Al-Homidan, 2004). Since dust may be a carrier of *Salmonella*, and one of the primary causes of dust generation in poultry house is motor activity of poultry which is directly affected by the parameter of lighting program. It will be a worthwhile effort to investigate the relationship existing between lighting system and incidence of *Salmonella* among poultry flocks as one of the technique for controlling prevalence of *Salmonella* in poultry. The results of multistate investigation of the relationships between lighting systems in commercial poultry house and prevalence of *Salmonella* contamination in poultry carcass have suggested that lighting system plays a role in the spread of the pathogen among birds of the same flock (Volkova, et al., 2010).

Movement of birds to different parts of the pen will obviously be affected by the lighting systems. Birds that were reared under high light intensity will be more active, in walking, preening, and forage behaviors (Alvino, et al., 2009; Blatchford, et al., 2012; Blatchford, et al., 2009; Boshouwers and Nicaise, 1993; Deep, et al., 2012; Martin, 1989; Newberry, et al., 1988). Presence of few *Salmonella* infected birds in a poultry flock reared under high light intensities might result to infection of more birds in the flock over time. Birds reared under high light intensity will be stimulated to more active including moving to different areas of facilities and increase feed consumption. Hence *Salmonella* infected birds in the flock might contaminate more locations of the pen that were not previously contaminated which could increase the risk of horizontal transmission of *Salmonella*. Preening activities will also increase the population of the

Salmonella in the intestine of infected birds, by ingesting the organism in the cloaca, similarly preening may also lead to continuous reinfection of the birds that are shedding the pathogen in the ceca and feces.

Since lighting intensity might affect the litter contrast, more foraging behavior will likely be exhibited in the flock reared under high intensity. Therefore, more of the *Salmonella* that were shed in fecal and ceca dropping will be ingested. Martin (1989) suggested that increased light intensity resulted to increased floor/ litter pecking. Light intensities may have linear relation with the number of birds infected with the pathogen, and the population of the pathogen in the infected birds. Other activities such as litter scratching, dustbathing and wing flapping might play a role in the distribution of *Salmonella* in the litter. In summary, the bird activities that are affected by light programs will directly or indirectly lead to increase in the distribution of *Salmonella* in poultry pen. Efforts to design a lighting system to minimize motor activity and not reduce production parameters of birds might have effect on the prevalence and concentration of *Salmonella* infection in poultry.

Another mechanism in which lighting system may promote *Salmonella* transmission in poultry could be stress (Freeman, et al., 1981; Huth and Archer, 2015; Kirby and Froman, 1991; Lien, et al., 2007; Xie, et al., 2008). Several studies have suggested that manipulative lighting parameters may have effect on stress levels and immune system experienced in birds. For example, Freeman et al. (1981) reported that birds reared under continuous lighting system from hatching to 3 wk of age were more stressed in comparison to birds that were reared under 12L:12D. Similarly, immature cockerel

reared under different lighting regimens of either 24L:0D or 12L:12D responded differently to injected antigens. The humoral immune response of the birds reared under 24 h photophase was significantly lower than in birds reared under 12 h photophase when exposed to the same antigen.

In another study, the colors of light under which birds were reared were shown to have impact on the immune status of birds (Xie, et al., 2008), birds reared under either green or blue light had significantly higher proliferation of blood T lymphocyte than those of the birds under red color light. The study also suggested that the anti-Newcastle disease serum was significantly higher in birds reared under green light than those reared under red light. Furthermore, the humoral immune response to antigen in birds reared under blue light was significantly higher than those of birds reared under red light.

However, Blatchford and co-workers (2009) reported that there was no significant difference in immune response to antigens by birds reared under 5 lux, 50 lux and 200 lux, although the trend of IgM titer response was numerically highest in birds reared under 50 lux and the lowest response was observed in the birds reared under 5 lux.

Since lighting programs have been associated with increase stress level (Freeman, et al., 1981) and affect immune status of birds (Kirby and Froman, 1991; Xie, et al., 2008). An environmental factor such as stocking density is known to increase stress and horizontal transmission of *Salmonella* in birds (Nakamura, et al., 1994a).

The source lighting may also increase stress level in birds, this was exemplified in a study where Light Emitting Diodes (LED) and Compact Fluorescent Lamp (CFL) were used as source of lighting in poultry production cycle. The assessment of stress in birds

measuring heterophil/ lymphocyte ratio, plasma corticosterone concentration and composite physical asymmetric suggested that lesser stress was observed in birds reared with Once LED light in comparison with other light sources (Huth and Archer, 2015).

Stress has been associated with increase in shedding and horizontal transmission of *Salmonella* among chicken (Nakamura, et al., 1994a), also Stress is implicated as one of the factors that adversely affect immune response in animals (Griffin, 1989; Moberg, 2000; Selye, 1936). Therefore, lighting program may play a role in the *Salmonella* infection status of birds and this suggestion is supported by the findings of Volkova and co-workers (2010).

Heat management in the intensive chicken farm

Poultry are homeotherms, and like all other homeothermic animals, poultry can maintain a fairly constant body temperature irrespective of the temperature of their surroundings. Since poultry maintain thermal homeostasis, they tend to loss excess heat generated to the environment through evaporation, conduction, convention and radiation (Elkheir, et al., 2008). The thermal requirement of poultry varies with age, as chicken grow older, the environmental thermal requirement reduce (Osbaldiston and Sainsbury, 1963; Soerjadi, et al., 1979). When the temperature of poultry environment is beyond the required temperature, the birds tend to loss or attempt to gain more heat either by using the normal mechanisms of heat transfer from the innermost body to the body surface and to the environment. And when the temperature gradient between the bird and the environment is low, birds use other mechanism such as thermal polypnea also known

as panting to increase rate of heat dissipation to the surrounding. On the other hand, poultry tend to generate and conserve more heat when the environmental temperature is below their body temperature. Therefore, appropriate environmental temperature is required for optimal metabolic activities of poultry. For instance, optimal performance of birds occurred when the environmental temperature is within the thermal neutral zone (Howlider and Rose, 1989; Washburn, 1985). When the temperature range is within the acceptable limit, and all other management requirements are also met the bird performance will be at its optimum level and this will translate to higher productivity (Howlider and Rose, 1987; Howlider and Rose, 1989; Washburn, 1985). Otherwise most of the energy that poultry supposed to use for muscle building or egg laying will be used for thermolysis or thermogenesis. Aside the poor production performance that will be observed in the poultry that was reared under an inadequate environmental temperature, there will be reduction in the feeding intake (Al-Fataftah and Abu-Dieyeh, 2007; Dale and Fuller, 1980; Mashaly, et al., 2004; Quinteiro-Filho, et al., 2012a). A study on the effect of environmental temperature on egg laying hen suggested that heat stressed birds body weight, feed intake and egg quality were adversely affected in comparison to the control hens (Mashaly, et al., 2004).

It is obvious that high environmental temperature in poultry pens could cause a lot of discomfort to chickens, which will result to distressed birds. Apart from the productivity performance of birds that is negatively affected by heat stress. The welfare of the birds in the pens with unfavorable environmental condition may be in jeopardy (Brambell, 1970). Heat stressed birds may experience physiological challenges that might

compromise their health. Studies have shown that animals in a distress situation will experience abnormal endocrine and neurological activities (Cannon, et al., 1929; Selye, 1936). The anomaly in the activities of the glandular system of animals in distress situation such as heat stress will lead to adverse effects on the homeostasis of the birds. Generally, the health conditions of the birds reared in temperature conditions that is beyond the thermoneutral zone are expected to deteriorate. And some of the metabolic characteristics of chickens that are negatively affected by heat stress may include physical behavior, productivity, immune function and digestive organs (Siegel, 1995) and the distortion of organs/ system might increase the susceptibility of birds to infection by pathogens such as *Salmonella*. Thereby increasing the risk of consumer to being infected with *Salmonella* due to eating of poultry products was contaminated with human foodborne pathogens.

Effect of heat on physiology of chicken

The optimal body temperature of chicken is about 41 °C and is maintained by either losing excessive heat generated to the environment or conserving the heat generated in the environment. Poultry response by displaying certain behavioral characteristics that enables them to maintain this temperature. In addition, feathers will be rearranged, increase in panting, wings and legs will be spread away from the body to allow the maximum surface area for heat loss (Siegel, 1995). One study suggested that heat stressed hens had a lower feed intake, spent more time drinking and panting irrespective of the strain of hens than the hens reared under normal ambient temperature (Mack, et

al., 2013). All these behaviors enable increase in heat dissipation to the surrounding environment. This differs in lower environmental temperature where the heat conservation behavior will be displayed by the chicken to maintain their body temperature. Both the behaviors displayed by chicken for heat conservation and heat loss require metabolic energy. Hence optimal productivity is achieved in the chickens reared in a thermoneutral zone (Osbaldiston and Sainsbury, 1963).

Physiological response to heat stress in chickens

Birds response to all form of environmental stressor are similar (Mcfarlane and Curtis, 1989). Studies have indicated that responses to different stressors are specific. The pattern of neurohormonal stress responses varies with the stressor (Mason, 1974; Seggie and Brown, 1982) but depend on its severity (Siegel, 1995). Stress responses in animals usually follow either hypothalamus-sympathetic nervous system pathway and results in the production of catecholamine and is mostly observed in the short term duration of stress (Cannon, et al., 1929) or the hypothalamus pituitary adrenal gland pathway (Selye, 1936). Both stress responses stimulate endocrine changes that have cascade of effect on the physiology of the animal, however the hypothalamus-pituitary-adrenal gland (HPA) axis response is attributed to exposure to chronic stress (Holmes, 1976).

Exposure to heat stress tends to disrupt the endocrine system in birds was illustrated in a study in which male 28 d old chickens were exposed to elevated environmental temperature of 30 °C for 2 wk and resulted in a 90 % increase in corticosterone level.

Also decrease of 52 % in tri-iodothyronine and 37 % thyroxine concentration in circulating plasma were observed in comparison to the control (Garriga, et al., 2006). Similarly, heat stress on layers causes an increase in the level of plasma corticosterone whereas the tri-iodothyronine concentration level decreased in the circulating plasma (Star, et al., 2008).

Another characteristic of heat stressed birds that could be negatively affected in heat stress bird is the immune system. Numerous studies have suggested that birds reared under high environmental temperature might be immunosuppressed. This implies that such birds will be more prone to disease infection than birds that are reared under normal ambient temperature. The mechanism in which high exposure to high temperature lowers immune response varies, studies have revealed that bird experiencing stress episode may undergo lymphocytosis (Gross, et al., 1980; Scanes, 2016), this situation resulted to decrease in the lymphocyte cells in the circulatory system. For example, a study on male birds showed that pre-heat stressed birds produced high antibody titer to antigens, but post- heat stressed birds produced significantly lower antibody compared to the control birds (Smith, 2003; Thaxton and Siegel, 1970).

Furthermore, consequences of heat stress on immune response in birds have also been attributed to the depression of the lymphoid tissues (Quinteiro-Filho, et al., 2010; Smith, 2003). Heat stressed birds had higher plasma corticosterone concentrations and decrease in the thymus, spleen, bursar of fabricus and liver weights. These organs are responsible for either the production, storage or the maturation of the lymphocyte cells which are decreased in weight because of an increase in the circulating plasma

corticosterone might explain the reason for the lower level of lymphocyte cells in the physiologically stressed animals. The result of the studies also indicated that there was a decrease in the macrophages basal oxidative burst in heat stressed (31 °C) birds.

The effect of heat stress on immunological cells seems to be cell specific, another study indicated that total leukocyte and lymphocyte cell count were decreased, the weight of the adrenal gland and bursar of fabricus were unaffected but the percentage of heterophil increased in the birds that were subjected to high environmental temperature over period of 2 h (Chancellor and Glick, 1960). This report did not agree with some of the other reports stated earlier on in this section in respect of the effect heat stress on bursar of fabricus. It is important to point out that there was a difference in age of the birds and duration of the heat stress was shorter than in the other studies, might have impact on the results. The effect of heat stress on the percentage of heterophil was in agreement with other reports on the effect of stress on immune response (Mcfarlane and Curtis, 1989). The result also indicated that the weight of adrenal gland was not affected in 14 d old heat stressed birds. Natural Killer (NK) cells are one of the innate immune cells that offer protection to the host against invading organisms and destruction of the infected host cells. The count and effectiveness of NK diminished in the animal exposed to stressful situation (Zorrilla, et al., 2001).

Heat stress effect on chicken resistance to Salmonella *infection*

Several factors are responsible for host susceptibility to *Salmonella* infection which may include the concentration of the pathogen, strain of the pathogen, route of infection,

immune status of the host, breed of the host, and age of the host (Grimont, et al., 2000). Other factors that have increased the susceptibility of chicken to *Salmonella* infection are physiological status, health and disease status and environmental stress (Bailey, 1993). While several literatures have indicated that the exposure of poultry to heat stress or any other stress resulted to lower feed intake, high mortality rate, reduced productivities, endocrine disruption and immunodulation. It should be noted that cumulative effect of the metabolic and physiological effect of stress might also increase the susceptibility of chicken to *Salmonella* infection.

Lowered feed intake might increase the susceptibility of animals to infection. The concept of lower immune responses due to a reduced feed intake described the prioritization in the allocation of nutrients to neural tissues, visceral tissues, bone, muscle and adipose tissue are supplied nutrient in the order (Hammond, 1952). The report suggested that in an animal with lower available nutrients, these tissues will be supplied with nutrient for their metabolic activities prior to order tissues. Therefore, immune cells may be lacking in the nutrients required for their metabolic activities in stressed animals, especially when feed intake is low.

Humoral and cell mediated immune response were low in heat stressed poultry (Scanes, 2016; Smith, 2003; Thaxton and Siegel, 1970), heat stress reduced poultry ability to fight off infectious agents such as *Salmonella*. In unstressed birds, *Salmonella* infection reduced in frequency before they attained market age (Bailey, 1993). Rearing of poultry in an environment that elicit physiological stress response will not only have

adverse effect on poultry productivity, health and welfare, the safety of poultry after harvesting will also be affected.

The food safety concern of heat stressed animal was further supported in an ex vivo study on the effect of heat stress on the ability of *Salmonella* Enteritidis to attach to intestinal tissue. The study suggested that there was an increase of 0.27 $\log_{10}$ CFU of *Salmonella* that attached to the ileal tissue of the 44 d of age birds that were exposed to heat stress of 30 °C for 24 h prior to been euthanized in comparison to the birds reared under normal environmental temperature of 23 °C (Burkholder, et al., 2008). Intestinal microbiota of heat stress poultry may be negatively affected thereby decreasing competition with *Salmonella* for colonization of the intestine (Bailey, 1988). The reduction in the competitive exclusion in poultry exposed to heat stress or any other stress such as feed withdrawal was also demonstrated in a study that heat stress modulated the microbial diversity of the intestine birds (Burkholder, et al., 2008).

Other studies have also indicated that expression of neuroendocrine hormone might be one of the factors that increased the susceptibility of stressed birds to pathogenic infection. Some of the findings in the studies were that the population and the expression of virulence factors by some gram negative bacteria increased in the presence of norepinephrine (Lyte and Ernst, 1992; Rahman, et al., 2000). Also when *Salmonella* Typhimurium was grown in a microbial cultural medium supplemented with norepinephrine (5×10^{-5} M/ mL), there was tenfold increase in the growth of the *Salmonella* in comparison to the control. Further, the enterotoxin (one of the virulence factors) produced by the pathogen increased in two to eight fold (Rahman, et al., 2000).

In another study, it was shown that when different catecholamine (Dopamine, Epinephrine and Norepinephrine) was included in cultural media, an increase in the growth rate of strain of *E. coli*, *Yersinia enterocolitica* and *Pseudomonas aeruginosa* were observed. And the increased in the growth rate of bacterial correlated with increase in the concentration of the hormones (Lyte and Ernst, 1992). The growth of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* can be stimulated by norepinephrine and dopamine (Belay and Sonnenfeld, 2002). All these studies suggested that the presence of catecholamine will enhance the growth and expression of virulence factors in some enteric pathogens. Since stressed animals may have high level of catecholamine in the circulating plasma (Cannon, et al., 1929), the disruption in the endocrine system may be one of the main factors that increase the stressed animal susceptibility to infectious agents.

Exposure of bird to heat stress may result in the structural deformation of the intestinal epithelium (Burkholder, et al., 2008; Quinteiro-Filho, et al., 2012a; Quinteiro-Filho, et al., 2012b) which could reduce the intestinal barrier function. Studies on mammalian model suggested that induced stress resulted to increase in the epithelial permeability in the entire gastrointestinal tract as a result adrenal corticosteroid (Meddings and Swain, 2000). Another study reported that rats exposed to different levels of stress for 4 wk had compromised epithelial-endothelial cells (Wilson and Baldwin, 1999). Intestinal epithelial cells function as an exchange barrier that selectively allow passage of certain molecule into the mucosa and the loss of the epithelial integrity might result to the passage of unwanted substance that might elicit inflammation in the host

(Lewis and McKay, 2009). Therefore, heat stress or any other form stress may result in the passage of pathogens or their enterotoxin into the intestinal lumen thereby resulting to system infection in heat stressed birds.

CHAPTER III

EVALUATION OF A COMMERCIAL PROBIOTIC PRODUCT IN CONTROLLING TRANSMISSSION OF *SALMONELLA* FROM LAYING HENS TO EGGS

Introduction

Human foodborne *Salmonella* infection has been identified as one of the major public health concerns in the United States per the results of epidemiological studies which suggest that *Salmonella* spp. contamination of food accounts for 11 % of annual foodborne illnesses in the country (Scallan, et al., 2011). Eating egg or egg containing products has been implicated as one of the vehicles for foodborne salmonellosis (Braden, 2006; De Buck, et al., 2004; Mishu, et al., 1991; Patrick, et al., 2004). Reports on the investigation into the mechanism of shell egg contamination revealed that a *Salmonella* infected layer hen could transmit the pathogen to the forming egg (De Reu, et al., 2006; Okamura, et al., 2001). These studies indicated that strategies for controlling shell egg contamination with *Salmonella* should also include prevention of laying hens infection with the pathogen.

One strategy to control *Salmonella* infection in egg laying flocks is vaccination. Laying hens that are vaccinated against *Salmonella* are more likely to lay eggs that are free of *Salmonella* (Cogan and Humphrey, 2003; Davies and Breslin, 2004) versus non-vaccinated birds. While vaccination of hens against *Salmonella* is an efficient strategy

for *Salmonella* control, its efficacy may require the identification of the targeted serotype (De Buck, et al., 2004). Therefore, there is a need for interventions that will protect layers from *Salmonella* infection irrespective of the pathogen serotype. Different types of antibiotics have also been used in the poultry industry at sub-therapeutic levels either for disease control or as a growth promoter. For instance, it was reported that inclusion of 200 g oxytetracycline per ton of chicken feed fed resulted in reduction of the *Salmonella* colonization of the intestine as well as lowered fecal shedding of *Salmonella* (Evangelisti, et al., 1975). Similarly, supplementation of poultry feed with sub-therapeutic level of oxytetracycline and neomycin reduced intestinal colonization, fecal shedding and prevalence of *Salmonella* Typhimurium in chicken flocks (Girard, et al., 1976). Some of the other antibiotics which have reduced the susceptibility of poultry to *Salmonella* infection are Salinomycin, flavophospholipol, polymyxin B, trimethoprim and enrofloxacin (Bolder, et al., 1999; Goodnough and Johnson, 1991; Seo, et al., 2000).

Despite the efficacies of various antibiotics in protecting poultry from infection with *Salmonella* and other infectious agents, the perceived risk of emergence of antibiotic resistance bacteria in the food chain has increased the need for other *Salmonella* control strategies in poultry. Even though there was little connection between animal fed antibiotics and human pathogen resistance to drugs, consumer perception of antibiotics causing resistance has led to decreased usage of antibiotics in the feed. Currently, antibiotics are being removed from the feed in layers. Several interventions such as drinking water acidification, feeding with probiotics (Nurmi and Rantala, 1973), prebiotics (Fukata, et al., 1999), synbiotics (Fukata, et al., 1999), experimental chlorate

product (Byrd, et al., 2008) short chain fatty acid (Van Immerseel, 2007) and other measures are being applied as alternatives to antibiotics. While most of these strategies have been shown to successfully reduce susceptibility of poultry to *Salmonella* infection, studies that investigated the control of *Salmonella* transmission from the laying hens to egg are still few and with variable results. Therefore, there is an urgent need to develop new strategies that will protect forming eggs from *Salmonella* contamination.

Supplementation of layer feed with probiotics may be a viable measure to control forming egg contamination with *Salmonella* since some probiotics may inhibit the ability of pathogens to colonize the intestine of poultry (Carter, et al., 2009; Garriga, et al., 1998; Pascual, et al., 1999). Since intestinal colonization is required before systemic and reproductive systems could be infected, feeding a probiotic to layers may be an effective mechanism to control *Salmonella* contamination of the egg (Gantois, et al., 2009). Studies have suggested that probiotics could stimulate an immune response in laying hens (Panda, et al., 2003). Other modes of probiotic action have also been attributed to inhibiting *Salmonella* colonization of the epithelial mucosa. For instance, some *Lactobacilli* species competitively lower the attachment of *Salmonella* to the ileal epithelial cell (Jin, et al., 1996b; Miyamoto, et al., 2000). Probiotics could also offer protection against pathogens infection in chickens through production of antibiotics, hydrogen peroxide, acid, bacteriocins and diacetyl (Jay, et al., 2005; Patterson and Burkholder, 2003). Also, immunomodulation of the chicken immune system could also be a mechanism of protection against pathogenic infection (Koenen, et al., 2004; Panda, et al., 2003). For instance, supplementation of the feed to 64 wk old leghorn hens with

commercially available probiotics that contain *L. acidophilus, L. casie, Bifidobacterium bifidum, Aspergillus oryzae, Streptococcus faceium and Torulopsis* spp. significantly increased humoral and cell mediated response to antigens (Panda, et al., 2003). In another report, the inclusion of layer feed with strains of *Lactobacilli* resulted in increased specific and nonspecific humoral responses to antigens. In addition, there was a decrease in the pH of the crop of the layer fed with probiotics and the intestinal microbiota was also modulated by the probiotics (Koenen, et al., 2004). Since all the available information have suggested that feeding poultry with probiotics may confer protection against *Salmonella* infection, *Lactobacilli* may prevent forming egg infection with *Salmonella* (Garriga, et al., 1998; Gusils, et al., 1999a; Vilà, et al., 2009).

The lactobacillus strain that was isolated for the commercially available probiotic product was identified as *Lactobacillus animalis* KCTC 3501. *Lactobacillus animalis* has a lot of metabolic similarities with *L. acidophilus* and *L. ruminis* (Dent and Williams, 1982), *L. animalis* is a homofermentative *lactobacillus*, that produce L (+) lactic acid isomer as a predominant metabolite (Dent and Williams, 1982). *L. animalis* is a highly auto aggregative and co-aggregative organism. This specie of *Lactobacillus* resists the acidity and bile salt in the gastrointestinal tract and can adhere strongly to the epithelial cell when compared to other broiler alimentary canal isolates (Akoy, 2015). These characteristics of *Lactobacillus animalis* enable it to qualify as a probiotic based on the definition and descriptions of probiotics (Fuller, 1989b; Havenaar and Huis, 1992; Jay, et al., 2005; Jin, et al., 1997).

The overall goal of this study was to determine if a defined probiotic (commercial probiotic product) fed to *Salmonella* Enteritidis orally challenged birds decreased *Salmonella* prevalence on eggs. Specifically, the study assessed the following:

- Efficacy of the probiotic in preventing intestinal colonization by *Salmonella*
- Ability of the probiotic to prevent systemic and reproductive organ infection with the pathogen
- Effect of the probiotic on ceca shedding of *Salmonella* by the hens

Materials and methods

Birds' procurement and assignment to cages and feeding design

Sixty (60) non-*Salmonella* vaccinated 16 wk old Hy-Line W-36 commercial pullets were purchased from the poultry farm managed by the Department of Poultry Science, Texas A&M University, College Station, Texas. The pullets were transported to Southern Plains Agricultural Research Center in Bryan, TX where they were divided into 2 groups and screened for *Salmonella* infection prior to the beginning of the study. Each group contained thirty pullets; one of the groups was designated as a control (n = 30), and the other group was the treatment (n = 30). All the birds were treated in compliance with the Animal Care and Use Committee (ACUC) requirement of USDA. Each group of birds was housed in individual cages in different rooms. Control birds were fed with a standard poultry industry layer diet (Leeson and Summers, 2005) while birds in the treatment group were fed the same standard poultry industry layer diet

supplemented with direct fed probiotic product that contained *Lactobacillus animalis* in the ratio 2.6:1 g/kg of probiotic product to feed as directed by the probiotic product manufacturer. The entire study lasted for six months. The feed for each group was prepared and replaced every 42 d to ensure the feed was fresh and the direct fed probiotics in the treatment feed was viable. Feed and water were supplied *ad libithum* to birds in both the control and the treatment groups.

Oral challenge of birds with *Salmonella*

After a two-week acclimation period during which each group was fed their respective diets, all birds were orally challenged with 3 mL (9.99 $\log_{10}$ CFU) of *Salmonella* Enteritidis (phage type 13A) that had been previously selected for resistance to Novobiocin (NO) and Nalidixic Acid (NA). The culture was prepared as described in Byrd et al. (2008). In summary the organism was thawed and 10 µL loopful of the pathogen was transferred into 10 mL of Tryptic Soy Broth (TSB) + 25 µg of NO and 20 µg of NA. The TSB culture was incubated at 37 °C for 8 h, a 10 µL of culture was transferred to sterile 10 mL of TSB and was incubated for 8 h at 37 °C and finally a 10 µL of *Salmonella* Enteritidis culture was transferred into a 400 mL of TSB. The *Salmonella* culture was then incubated at 37 °C for 8 h. The birds were repeatedly challenged with the *Salmonella* Enteritidis every 6 wk; therefore, the birds were orally challenged with the pathogen 4 times during the study.

Microbiological analyses

Sampling of feed, bird, egg and ceca

To determine the amount of the probiotic that was fed to the birds, three samples of the probiotic product, control feed and five samples of the treatment feed were collected on the days when the feed were mixed and repeated on a biweekly basis. Each of the feed samples was collected from different parts of the container (top, middle and bottom) to ensure that actual estimate of the *L. animalis* content in the feed was accurate. In addition, prior to sampling of eggs and ceca contents for *Salmonella*, five birds from both the control and the treatment groups were euthanized. The ceca were retrieved and analyzed for the presence of *L. animalis* in the probiotics. Once a week cecal contents were collected from both groups and pooled separately to be analyzed for the presence and population of *Salmonella* which colonized the gastrointestinal tract of the birds. Also a mean of 21 eggs and 22 eggs were aseptically collected twice per week from control and treatment group respectively for microbial analysis. The shell and liquid content of the eggs were tested for the presence of *Salmonella*. The feed, ceca and egg sample collection were repeated for six months. At the end of the study, the remaining 25 birds in each group were euthanized and necropsied. Their liver-spleen, ovary and ceca were tested for the presence and population of *Salmonella* as described below.

Analysis of egg, ceca and hen for Salmonella

Egg. The crush and rub method (Musgrove, et al., 2005) was modified in the preparation of egg shells for *Salmonella* assay. Briefly, each egg sample was aseptically

cracked opened on the edge of a sterile beaker and the egg liquid (internal content) was emptied into a sterile bag. The egg shell inner cavity was rinsed with PBS to ensure removal any adhering albumen. The shell with its membrane was crushed and transferred into a sterile 50 mL disposable centrifuge tube. Ten mL of buffered peptone water (BPW) was added to the crushed shell and membrane in the centrifuge tube. A sterile rod was used to further crush the shell and its membrane by continuously pounding for 1 min. The pulverized shell and its membrane were pre-enriched with BPW then incubated at 37 °C for 24 h, the pre-enriched sample (0.1 mL) was transferred into 10 mL of Rappaport Vassiliadis (RV) and incubated at 42 °C for 24 h. A 10 µL loopful of the enriched media from the shell and its membrane was streaked on the surface of Xylose-Lysine-Tergitol 4 (XLT4, supplemented with NA and NO) agar. Samples were incubated at 37 °C for 24 h and were observed for typical *Salmonella* colony.

The egg liquid in the sterile bag was homogenized and 50 g of the egg liquid homogenate was added to 5 mL of 10 X BPW (ratio 10:1 mass/volume). The mixture was homogenized continuously for 1 min and incubated at 37 °C for 24 h. The pre-enriched egg liquid (0.1 m L) was sub-cultured into 10 mL of RV and was incubated at 42 °C for 24 h. A 10 µL loopful of the enriched egg liquid was streaked onto XLT4 agar. The plates were incubated at 37 °C for 24 h and observed for typical *Salmonella* colony morphology.

Ceca content. Each cecal content weighing 1 g was diluted in 9 ml of PBS, 1 mL of the diluted ceca content was serially diluted in 9 mL of PBS and 0.1 mL of each of the

serially diluted sample was spread on the surface of XLT4 agar. Also the prevalence of *Salmonella* was determined by transferring 1 g of the ceca content into 9 mL of BPW and was pre-enriched at 37 °C for 24 h. The preenriched cecum (0.2 mL) was enriched in 20 mL of RV and incubated at 42 °C for 24 h. A 10 µL loopful of the enriched ceca content was streaked on XLT4, for the detection of *Salmonella* in the sample and incubated at 37 °C for 24 h and typical *Salmonella* colonies were enumerated and observed respectively.

Organs. Cecum, liver-spleen and ovary of each bird were pre-enriched in 9 mL, 13 mL and 34.2 mL of BPW respectively and were incubated at 37 °C for 24 h. A 0.2 mL of the enriched BPW from the pre-enriched organs were transferred into 20 mL of RV and incubated at 42 °C for 24 h. And a 10 µL loopful of the enriched samples were streaked on XLT4 (supplemented with NA and NO) and incubated at 37 °C for 24 h. In addition, 0.25 g of the content of the other cecum was emptied into 2.25 mL of PBS, homogenized and 1 mL of the ceca content dilution was serially diluted in 9 mL of PBS. Furthermore 0.1 mL of the serial dilution sample was spread on the surface of XLT4 (containing NA and NO) agar and were incubated at 37 °C for 24 h. The typical morphology of *Salmonella* colonies was observed and enumerated.

Polymerase Chain Reaction (PCR) assay for detection of Lactobacillus animalis *(probiotic)*

Feed and product culturing, extraction and purification of colonies DNA. Two sets of feed samples for the control and the treatment group plus the remaining probiotic

were enumerated for viable *L. animalis*. Each sample was prepared by transferring 10 g of feed or the probiotic into a sterile bag containing 90 mL of Phosphate Buffered Saline (PBS) and the suspension was thoroughly mixed by hand massaging for 1 min. One mL of the mixture was used to make serial dilutions in 9 mL of PBS. Then 0.1 mL of the serially diluted sample was transferred and spread on Lactobacilli MRS agar (Becton, Dickson and Company, Franklin, NJ). The bacterial cultured MRS agar plates were anaerobically (Abdulamir, et al., 2010) incubated at 37 °C for 24 h. Five distinct bacteria colonies from each sample plate set were isolated and streaked on new MRS agar plates to determine if a pure cure was isolated. The purified colonies were prepared for identification with PCR assay by extracting the DNA of the colonies using UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, Ca) as described in the instruction manual. The DNA samples were stored at -20 °C for further use in PCR and sequencing analysis.

Lactobacillus *isolation, DNA extraction and purification from ceca content of sampled birds.* Five birds from both the control and the treatment birds were euthanized by exposure to CO_2 and confirmation by cervical dislocation. The cadavers were disinfected, necropsied and ceca were retrieved. Cecum contents of each bird were used to prepare DNA samples that were used for pyrosequencing analysis. A 0.25g of the cecum content was diluted with 2.25 mL of PBS, 1 mL of the dilution was used for serial dilution in 9 mL of PBS, and 0.1 mL of the serially diluted samples were spread on MRS agar plates and were incubated anaerobically at 37 °C for 24 h. Five colonies from each bird sample plate were isolated, streaked on MRS agar plates and incubated under

anaerobic condition at 37 °C for 24 h. DNA of the colonies from the streaked plates was extracted and purified in the UltraClean Microbial DNA Isolation Kit as described below and were used for PCR analysis.

Polymerase chain reaction assay, gel electrophoresis and purification. Primers sequence – Lacto- 16SF 5'- CGC TTT ACG CCC AAT AAA TCC GG- 3' and Lacto- 16SR- 5'- CGC TTT ACG CCC AAT AAA TCC GG- 3' (Abdulamir, et al., 2010; Abed, 2013) were synthesized and supplied by Integrated DNA Technologies, Coralville, AI. Each of the primer was dissolved in Rnase/ Dnase free water to achieve the primer concentration of 100 pmol/µL and stored at -20 °C. A final concentration of 10 pmol/ µL was used in the PCR reaction. Amplification was performed using 2x Phusion High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Waltham, MA) and 3 µL of the DNA sample. DNA amplification was conducted in the DNA Engine, Peltier Thermal Cycler (Bio- Rad Laboratories, Inc. Hercules, CA). As described in Abed, 2013, the DNA amplification was obtained in 40 cycles with temperature profiles of 95 °C for 3 min for the initial denaturation of the DNA double strand. Subsequently, 40 cycles with each cycle at 95 °C for 30 s was used to denature double strand per cycle. Single stranded DNA was annealed to the primers at 61 °C for 40 s, while DNA extension occurred at 72 °C for 1 min with a final elongation at 72°C at 5 min. Samples were held at 4 °C. PCR samples were separated on electrophoresis 1 % (w/v) agarose gel containing ethidium bromide. A mixture of 3 µL of the loading buffer and 6 µL of the PCR samples were loaded onto agarose gel. A 100 bp DNA Ladder Standard (New England Biolabs Inc. Ipswich, MA) was used to determine the base pairs molecular

weight of the samples, and 100 V was passed through the apparatus for 2 h for fragmentation of the contents of the PCR samples. Subsequent migration of these PCR samples was measured and compared under UV light in Multiimage Light Cabinet Filter Position (Alpha Innotech, San Leandro, CA). PCR samples were then purified with QIAquick PCR purification Kit (Qiagen, Hilden, Germany) following the manufacturer's guideline. Concentrations on purified PCR samples were determined using NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA) prior to shipment to a third party laboratory (Gene Technology Laboratory, College Station, TX) for DNA sequencing (Sanger sequencing). DNA sequence results were compared to the data base bank of the NCBI for the samples identification.

Extraction of DNA of the ceca microbiota and subsequent pyrosequencing analysis. The content of the other pair of the cecum of each bird was used for pyrosequencing assay. The DNA of all the organisms that was present in the cecum were extracted using QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) as described in the QIAamp DNA Stool Handbook. The eluted DNA samples were stored at -80 °C until they were shipped to the third party laboratory (Molecular Research (MRDNA), Shallowater, TX) for pyrosequencing analysis. At the Laboratory, the DNA samples were analyzed using the procedure described in www.mrdnalab.com. Briefly, the 16S rRNA gene V4 variable region PCR primers 515/806 with barcode on the forward primer were used in a 28 cycle PCR. HotStarTaq Plus Master Mix Kit (Qiagen, USA) was initially subjected to 94 °C for 3 min, and subsequently to 28 cycle with each cycle of 94 °C for 3 s, 53 °C for 40 s and 72 °C for 1 min, and a final elongation at 72 °C for 5

min. The amplicons were examined for their molecular weight in 2 % agarose gel, purified in calibrated Ampure XP beads and were used for the preparation of illumine DNA library. The DNA sequencing was performed with MiSeq methods (www.mrdnalab.com), the sequences were joined, depleted of barcodes and sequences with the following features removed (i) <150 bp, (ii) ambiguous base calls, (iii) chimeras removed. The sequences were denoised, the Operational Taxonomic Units (OTUs which is defined by clustering at 3 % divergence) were generated. The final OTUs were classified by BLASTn against a curated database of RDPII and NCBI.

Statistical analyses

The $\log_{10}$ CFU of *Salmonella* enumerated in the ceca content of the live birds, the $\log_{10}$ CFU of lactic acid bacteria content of the feed of the birds, and the $\log_{10}$ CFU of *Salmonella* content of the cecum after necropsy in both groups of samples were analyzed and compared for analysis of variance (ANOVA) using PROC GLM procedure of SAS version 9.4. Prior to conducting ANOVA test on the concentration of *Salmonella* in the samples (cecal droppings and cecal content), Levene's test was used to assess homogeneity of variance between the samples from the control fed and probiotic fed birds. The means of the samples that were significantly different when $P < 0.05$ were separated using Duncan's Multiple Range Test procedure of SAS version 9.4 (SAS Institute, Cary, NC). Also, the difference in the prevalence of *Salmonella* in egg shells, egg liquid, liver-spleen, ovary, ceca and cecal droppings between both groups of hens

were determined with either Chi Square or Fischer Exact Test using PROC FREQ procedure of SAS version 9.4. Also the significant difference was when $P < 0.05$

Results and discussion

Feed and bird lactic acid bacteria assay

The probiotic product supplemented feed was mixed in a ratio 2.6:1 g/kg of probiotic product to feed. There was a significant ($P = 0.0003$) difference in the population of lactic acid bacteria between the control feed (3.9 $\log_{10}$ CFU/ g) and feed supplemented with the probiotics (4.7 $\log_{10}$ CFU/ g). This analysis indicated that there was an increase of 0.8 $\log_{10}$ CFU/ g in the population of the lactic acid bacteria due to the inclusion of the probiotic to the layer feed. Meanwhile the concentration of *Lactobacillus* in the probiotic product was 7.9 $\log_{10}$ CFU/g, theoretically the difference in the population of lactic acid bacteria between the feeds was calculated to be about 5.3 $\log_{10}$ CFU/g instead of confirmed 0.8 $\log_{10}$ CFU/g of Lactobacillus. The reason for the disparity between these values remains unclear.

The DNA sequence of the lactic acid bacteria isolates of the probiotic product indicated that the isolates are mostly similar to a strain of bacteria identified as *Lactobacillus animalis* KCTC 3501, but all the isolates of both the control feed and probiotic supplemented feed were not similar to these probiotic product isolates (*Lactobacillus animalis* KCTC 3501). Similarly, none of the chicken ceca lactic acid bacteria isolates were similar to the probiotic isolates.

Furthermore, the results of the analysis of the ceca content microbiota also suggested that there was no *Lactobacilli* spp. that was similar to *Lactobacillus animalis* KCTC 3501 in the cecal of either the control fed or probiotic fed layers. In addition, there was no significant difference ($P > 0.05$) between the microbial diversity of the cecal content of both the probiotic fed and control feed fed layers. Studies have indicated that the efficacies of probiotics in controlling *Salmonella* infection in animals depend on its concentration and the characteristic of the probiotic organism (Lee, et al., 2000).

The differences in the inhibitory effect of probiotics on *Salmonella* control might also depend on the dose. And this might be further elucidated in different studies that applied same strain of *Saccharomyces boulardii* but in different concentration for *Salmonella* control in young broiler chicks. When ratio of 100:1 g/kg of *Saccharomyces boulardii* to feed was applied, the prevalence and the level of *Salmonella* infection were significantly reduced (Line, et al., 1998). But in a similar study when the concentration of *Saccharomyces boulardii* was in ratio 50:1 mg/kg of probiotic to feed, the prevalence and concentration of *Salmonella* in the ceca were not affected (Mountzouris, et al., 2015).

Therefore, higher concentration of daily intake of *Lactobacillus animalis* KCTC 3501 might be needed to effectively prevent intestinal colonization by *Salmonella*. Mechanisms of preventing *Salmonella* colonization might be due to either competition (Gusils, et al., 1999b; Jin, et al., 1996b; Nurmi and Rantala, 1973), immunodulation (Panda, et al., 2003), production of inhibitory metabolites (Axelsson, et al., 1989; Mishra and Lambert, 1996) and modulation of intestinal microbiota (Hosoi, et al., 2000;

Kleessen, et al., 2001) or combination of some or all of the mechanisms to inhibit growth and survival of pathogen in the gastrointestinal tract. The age of the birds treated with probiotic might also affect the ability of probiotic to actually prevent a pathogenic intestinal infection (Nurmi and Rantala, 1973). When the intestinal microbiota of bird is still developing, it might be easier for probiotics to have access to binding sites, but as birds grow older, the intestinal colonization become steady. Hence it may be difficult for any organisms that is been introduced to the gastro intestinal (GI) tract to colonize epithelial cells. For example, an in vitro study demonstrated the ability of *Lactobacilli* to competitively prevent adherence of *Salmonella* Typhimurium and *Salmonella* Pullorum to epithelial cells, but the *Lactobacilli* were unable to displace the pathogens from the epithelial cells (Jin, et al., 1996b). Therefore, the organisms in the GI tract may have advantages over organisms that are new to the environment. In this study, the efficiencies of the probiotic in controlling *Salmonella* infection in the hens might have negatively affected by the age of the birds when they were fed the probiotic.

Egg contamination with *Salmonella*

The control group had a total of 1085 eggs tested of which 26 (2.4 %) egg shells and 2 (0.2 %) egg liquid samples were contaminated with *Salmonella* resulting in a total of 2.6% (28/1085) eggs positive for *Salmonella*. A total of 1153 eggs were collected from the birds in the group fed with the probiotic product. Of these 1153 eggs, 20 (1.7 %) egg shells were *Salmonella* positive with none of the liquid internal content was positive for *Salmonella.* Although numerically, higher number of eggs from the control birds was

contaminated with *Salmonella* versus the treated, the difference in the number of the eggs contaminated with the pathogen between both groups of bird was not significantly different ($P > 0.05$). The detail of the proportion of egg contaminated with *Salmonella* in this study is shown in Table 1.

Table 1. Prevalence of *Salmonella* in eggs from both the control and probiotic fed hens

Group	Egg shell *Salmonella* positive/ Total (%)	Egg liquid *Salmonella* positive/ Total (%)
Control feed fed laying hens	26/1085[a] (2.4)	2/1085[a] (0.2)
Probiotic fed laying hens	20/1153[a] (1.7)	0/1153[a] (0)

Numbers with the same superscript letter a - b across the column are not significantly different $P > 0.05$.

Salmonella infection in hens

Even though not significantly different, the prevalence of *Salmonella* was 50 % among the birds fed the control diet and 36% in the birds fed the probiotic (Table 2). In the case of the ovary, only one bird ovary tested positive for *Salmonella* in the control, whereas the ovary of three birds was *Salmonella* positive among the probiotic fed birds. *Salmonella* was detected in 8 of the ceca in the birds fed the probiotic and 10 positive ceca were observed from the control feed. None of the liver-spleens of the birds fed with

the probiotic product were *Salmonella* positive, but the liver-spleens of two birds in the control group were *Salmonella* positive. The difference between the prevalence of *Salmonella* in the ovary, ceca and the liver-spleen of both group of bird was not significantly different ($P > 0.05$).

There was also no significant difference ($P > 0.05$) between the shedding of *Salmonella* from the ceca of control birds (3.0 $\log_{10}$ CFU/g) when compared to the treated group (2.8 $\log_{10}$ CFU/g) (Table 3). At the termination of the study, the mean of the *Salmonella* concentration in the cecal contents of the birds fed probiotics (1.0 $\log_{10}$ CFU/ g) was not significantly different from birds fed the control diet (1.2 $\log_{10}$ CFU/ g). In determining the mean concentration of *Salmonella* in the cecal of the birds in both

Table 2. Prevalence of *Salmonella* in the organs of the laying hens from both the control and probiotic fed groups

Group	Hen *Salmonella* positive/ Total (%)	Ovary *Salmonella* positive/ Total (%)	Ceca *Salmonella* positive/ Total (%)	Liver-spleen *Salmonella* positive/ Total (%)
Control feed fed laying hens	12/24[a] (50)	1/24[a] (4.2)	10/24[a] (41.7)	2/24[a] (8.3)
Probiotic fed laying hens	9/25[a] (36)	3/25[a] (12)	8/25[a] (32)	0/25[a] (0)

Numbers with the same superscript letter a - b across the column are not significantly different $P > 0.05$.

groups, the $\log_{10}$ of CFU of the cecal *Salmonella* concentration below detection limit was also included. To be specific, *Salmonella* concentration in the ceca of 17 and14 hens in the probiotic fed and control feed fed group was below detection limit respectively. Differences between the concentration of *Salmonella* in the cecal droppings and the ceca of the birds may be due to many factors. These may include the time interval between when the cecal droppings were egested by the birds and analyzed for *Salmonella*. Cross contamination of the cecal dropping by environment agents that were contaminated by *Salmonella* may affect the concentration of the pathogen in the ceca samples. Therefore, the concentration of the pathogen in the cecal dropping may not indicate the actual concentration of the pathogen in the ceca of the infected birds.

The infection of chicken with *Salmonella* varies depending on parameters such as the strain of the pathogen, age and immune status of the bird (Grimont, et al., 2000). Older birds (42 wk of age) such as those used in this study will be more resistant to *Salmonella* infection than young birds. The age of the bird may be the reason why the mean of the concentration of *Salmonella* in the ceca was low, despite the population of the pathogen (9.99 $\log_{10}$ CFU/ 3 mL) that was periodically used for oral challenging of each of the bird.

Table 3. The population of *Salmonella* that colonized the ceca content of the laying hens that were orally challenged with 9.99 log_{10} CFU/ mL *Salmonella* Enteritidis at 6 wk intervals prior to necropsy at 42 wk of age.

Group	Means of Log_{10} *Salmonella*/ CFU/g per layer ceca droppings	Means of Log_{10} *Salmonella*/ CFU/g per cecal contents	*Salmonella* detection in the layer ceca droppings (%)	*Salmonella* detection in the ceca content (%)
Normal feed fed layers	2.95 ± 0.24^a	1.24 ± 0.33^a	85.71	41.7
Probiotic fed layers	2.75 ± 0.17^a	1.00 ± 0.30^a	85.47	32

Numbers with the same superscript letter a - b across the column are not significantly different $P > 0.05$.

The concentration of the *Salmonella* below detection limit (< 2 log10 CFU/g) was included in the calculation of the means of the *Salmonella* population in either the cecal dropping or cecal content

Conclusion

Lactobacillus animalis was not found in the ceca of the hens fed with the probiotic supplemented feed in this study. Feeding probiotic to the layer birds at the concentration used in the study did not prevent *Salmonella* colonization in the liver-spleen, ceca and the ovary of the birds. *Salmonella* contamination of eggs was not controlled by the probiotic fed to the layer. Also the level and prevalence of *Salmonella* in cecal shedding and ceca were not reduced by the probiotic. In addition, the prevalence of *Salmonella* in

the liver-spleen, ovary and of hens were not impacted by feeding on probiotic supplemented feed in this study.

CHAPTER IV

IMPACT OF LIGHT INTENSITY ON THE HORIZONTAL TRANSMISSION OF *SALMONELLA* AMONG BIRDS IN THE SAME PEN

Introduction

Poultry and poultry products have been identified as one of the food commodities that are frequently associated with human foodborne *Salmonella* infection because they can become contaminated with non-typhoidal *Salmonella* serotypes (Food Safety and Inspection Services, 2009; FoodNet, 2010). Between 2010 and 2015, poultry food commodities were associated with about 30 % of human foodborne *Salmonella* infection outbreaks in the United States (Centers for Disease Control and Prevention, 2015). And 44 % of the human poultry borne *Salmonella* infection between this period (2010 to 2015) were due to contact with *Salmonella* infected live chicken. All these reports suggested the need for more robust *Salmonella* control strategy at the preharvest stage of poultry production.

While effective *Salmonella* control interventions such as vaccination, feed and water additives have been introduced to control *Salmonella* infection during live production, incidence of human poultry borne *Salmonella* infection has not been eliminated. Therefore, better approaches are still needed to control this pathogen in poultry. Consumers have increasingly favored the reduction or elimination of some poultry feed additives such as antibiotic growth promoters (AGP) that have been historically known

to be effective in the control of *Salmonella* infection in poultry. The concern for the development of antibiotics resistance pathogens due to the use of AGPs in poultry feed has increased the pressures on poultry farmers to seek alternatives. Although, reports have suggested that usage of antibiotics as feed additives in food animal production might not be responsible for the emergence of antibiotic resistance microorganisms, results of the survey on prevalence of antibiotic resistant organisms in cattle revealed that less than 4 % of human antimicrobial resistant pathogen are of food animal origin (Bywater, 2004). Study also indicated that some antibiotics resistant *Salmonella* Typhimurium isolated from chickens did not have a known history of contact with antibiotics (Evangelisti, et al., 1975).

Poultry production management practices may be exploited as a multi hurdle approach to controlling *Salmonella*. Decreasing stocking density has been shown to be effective in the reduction of horizontal transmission of *Salmonella*. This improvement was demonstrated in molted hens in cages that were 1 m apart from one another, these birds were challenged with *Salmonella* Enteritidis and the unchallenged birds were monitored for *Salmonella* infection. The result of the study suggested that 75 % of birds in adjacent cage to the challenged birds were infected with the pathogen between 3 to 8 d post challenge. Whereas only 25 % of the birds in alternate cages became infected with the pathogen after 10 d (Holt, et al., 1998). Light management in the poultry house environment has been shown to have an effect on the prevalence of *Salmonella* in birds. Multistate studies on the effect of lighting programs on prevalence of *Salmonella* in poultry carcasses after harvest suggested a positive correlation between the prevalence of

Salmonella and daily long hour reduced lighting (> 18 h) of reduced lighting in the last 1 wk of live production (Volkova, et al., 2010). The results indicated a reduction in the prevalence of *Salmonella* on the exterior of broiler, litter swabs in poultry houses and carcass at post chilling stage of harvesting in broilers reared under long hour.

Other studies have also suggested the effect of different parameters of lighting on the health and behavior of birds. The immune status of poultry reared under long period of photophase may be adversely affected. This was illustrated by (Kirby and Froman, 1991) where birds reared under 24 h of light had poor cell mediated and humoral immune response to antigen in comparison to birds reared under 12L:12D. In a similar study, young birds reared under long period of photophase experienced high level of physiological stress than birds of the same age reared under 12L:12D (Freeman, et al., 1981). The wavelength (color) of light used for rearing of birds may also have effect on the immune status of birds. Broilers reared under different colors of monochromatic light had variation in T cell proliferation and antibody production (Xie, et al., 2008). This study indicated that there was highest proliferation of T lymphocyte cell in the birds reared under green light, compared to the lowest proliferation of the cell in the birds reared under red light. The antibody titer production was lower in the birds reared under red light in comparison to the birds reared under either green or blue light. All these studies suggested that the features of lighting system management practice may affect the susceptibility of chicken to infection.

The motor activities of birds may also be affected by the characteristic of the light used in in the poultry houses. Lighting intensities can affect behaviors such as litter

pecking, higher frequency of litter pecking was recorded in the birds reared under high light intensity level (Martin, 1989). Blatchford, et al. (2009) reported that the motor activities increased in birds with increasing lighting intensities. The motor activities were the lowest in the birds reared under 5 lux when compared to the birds reared under 200 lux. Furthermore, the frequency of standing, walking and total motor activities of birds reared under 180 lux was higher than in birds reared under 6 lux (Newberry, et al., 1988).

Different lighting management practices may affect the dispersion of pathogenic organisms in poultry houses. The amount of dust generated in poultry house has been linked to either the lighting management practices or the activities of the animals (Al-Homidan, 2004; Calvet, et al., 2009; Ellen, et al., 2000). Dust has been identified as one of the vehicles of *Salmonella* distribution in poultry house (Harbaugh, et al., 2006; Marin, et al., 2011; Mitchell, et al., 2002). Other studies have also associated lighting system in poultry husbandry with prevalence of *Salmonella* on carcasses after harvest (Volkova, et al., 2010), and a lowered resistance to pathogenic infection (Xie, et al., 2008). Lighting parameters may increase stress level in birds (Huth and Archer, 2015; Prayitno, et al., 1994; Prayitno, et al., 1997). The stressed animals are generally immunocompromised (Cannon, et al., 1929; Selye, 1936), and are more susceptible to infection. However, more information is needed to understand the risk of *Salmonella* infection in birds due to manipulation of light parameters in poultry management.

Based on the previous studies exploring the effect of lighting system on the activities of birds, a hypothesis that susceptibility to *Salmonella* infection could also increase due

to fecal shedding of the pathogen and lower immune function during stress. Therefore, the goal of this study was to determine the effect of lighting intensities on broiler *Salmonella* infection. And the objectives of the study were as follows;

- To investigate the effect of 5 lux and 50 lux light intensity on transmission of *Salmonella* in broilers.
- To assess the effect of light intensity on *Salmonella* colonization of the ceca, and the prevalence in the crop, liver-spleen and ceca of broilers.
- To evaluate the effect of light intensity on physiological stress in birds.
- To determine the effect of light intensity on the motor activities of birds.

Materials and methods

Pen design and lighting

Two trials of the study were conducted and in each trial, 1 d of age Ross 708 broilers chicks (n = 100) were purchased from a commercial hatchery and were transported to the Southern Plains Agricultural Research Center. Prior to the start of the study, the birds were tested for the presence of *Salmonella*. The birds that tested negative to *Salmonella* were used and divided into 4 groups of 25 birds each (Table 4).

Table 4. Experimental design

Lighting intensities (Lux)	Control/ unchallenged birds	Treatment /challenged bird
5 (Treatment)	25	5 challenged out of 25
50 (Control)	25	5 challenged out of 25

On d 3, 5 birds per pen (seeder birds) were wing banded from each of the treatment/ challenged groups and were orally challenged with *Salmonella* Typhimurium. The remaining birds were left unchallenged and used as contact birds to determine horizontal transmission of *Salmonella*. From 2 to 10 d, the source of light was from fluorescence light, the lighting regimen in both rooms housing the birds were 23L:1D (photophase: scotophase) with the light intensity that ranged from 145 lux to 175 lux. From 3 d to the end of the study (20 d), the light source was changed to LED light, and the intensity of light in the room housing birds in the control group was adjusted 50 lux and to 5 lux in the treated group.

From 10 to 20 d of the study, the lighting schedule in both rooms was changed to 16L: 8D. The activities of birds in the pens of the *Salmonella* challenged birds were monitored with a motor sensor device, a passive infrared detector (PID) (Blatchford, et al., 2009; Nielsen, et al., 2003; Pedersen and Pedersen, 1995) that was connected to a programmed data-recording device and was positioned in both pens to scan the entire width and length of the pen. The mechanism of the operation of this motor sensor device has been described in Blatchford, et al. (2009). Briefly, any motor activity in the pen led

to the motor sensor turning off, and the sensor remained turned off until there was another movement in the pen. At a specified time, a data logger is recording if the device is on / off, and this information is used to determine the motor related activities of the birds in the pen during both photophase and scotophase (Blatchford, et al., 2009). At the end of the study all the birds in the challenged group, and the unchallenged 5 wing banded birds from each group were euthanized by exposure to CO_2 and confirmed with cervical dislocation (Leary, et al., 2013). The cadavers were disinfected and necropsied and the crop, liver-spleen and ceca were weighed and analyzed for *Salmonella*.

Culturing of *Salmonell*a and oral challenge of birds with *Salmonella*

A 10 µL of the pure culture of Novobiocin (NO, Sigma-Aldrich, St. Louis, MO) and Nalidixic (NA, Sigma-Aldrich, St. Louis, MO) resistant *Salmonella* Typhimurium stored in -80 C freezer was thawed, cultured in 10 mL of Tryptose Soy Broth (TSB, Becton, Dickson and Company, Franklin, NJ) that contained 25 µg and 20 µg of NO and NA respectively. And the culture was prepared as described in the studies on the effect of experimental chlorate product on broiler chicken (Byrd, et al., 2003; Byrd, et al., 2008). Then a 5 mL of PBS was inoculated with the suspension of *Salmonella* Typhimurium and the absorbance of the suspension was measured in a spectrophotometer (Spectronic 2OD by Milton Roy Company, Ivyland, PA) at wavelength of 625 nm. The absorbance level of the *Salmonella* suspension was adjusted by adding more of PBS until the absorbance level was equal to 1.58 (10^8 CFU/ mL of NO and NA resistant *Salmonella*

Typhimurium). The five wing banded birds (seeder birds) in each of the pens housing birds in the challenged groups were orally challenged with 7.7 $\log_{10}$ CFU/ mL of the NO and NA resistant *Salmonella* Typhimurium. While the remaining unchallenged 20 birds in each of the pens were the contact birds.

Analyses of blood samples for leukocyte and heterophil/lymphocyte ratio

On 10 and 20 d of the study, 3 mL of blood samples of all the wing banded birds were collected through the jugular vein. Sampled blood was immediately transferred into vacutainer EDTA 10 mL PK100 to prevent clotting (Zarnitsyna and Zhu, 2011), held at ambient temperature and used for total leukocyte and heterophil-lymphocyte ratio analyses. The concentration of the total leukocyte and heterophil-lymphocyte ratio content of blood were used as the indicators of physiological stress in the birds (Dhabhar, 2002; Dhabhar, et al., 1994; Gross and Siegel, 1983; Mcfarlane and Curtis, 1989).

The total leukocyte and heterophil-lymphocyte cells ratio were enumerated using the methods described in Natt, and Herrick, 1952; and Genovese et al. (1998). Briefly, a 10 µL of blood sample was transferred into sterile 2 mL centrifuge tube that contained 1000 µL of Natt and Herrick diluent. The blood diluent mixture was homogenized, 15 µL of the mixture was transferred to the hemocytometer and the total leukocyte cells were read under a light microscope. Heterophil-lymphocyte ratio was analyzed by making a smear of the blood sample on a slide, fixed and stained in Hema 3-stain (Shandon Scientific,

Pittsburgh, PA) (Genovese, et al., 1998), and each of the cells (heterophil and lymphocyte) was enumerated under a light microscope and the ratio was calculated.

Microbiological analyses

Screening of day old broiler chicks for Salmonella *infection*

Tray liners that were used to transport the broiler chicks from the hatchery were placed in a whirl pak bag, preenriched (Waltman and Gast, 2008) in 200 mL of Buffer Peptone Water (BPW, Becton, Dickson and Company, Franklin, NJ), hand massaged for 1 min and incubated at 37 °C for 24 h. A 0.2 mL of the preenriched culture was transferred into 20 mL of Rapport Vasiliadis (RV, Becton, Dickson and Company, Franklin, NJ) Broth and incubated at 42 °C for 24 h. Then, a 10 µL of the enriched sample was streaked in triplicate onto Xylose-Lysine-Tergitol 4 (XLT4, Hardy Diagnostic, Santa Maria, CA) agar. The plates were also incubated at 37 °C for 24 h and observed for growth of colonies that are typical of *Salmonella* morphology.

Determination of colonization of alimentary canal by Salmonella

The crop was preenriched in BPW at 37 °C for 24 h, and then 0.2 mL of the preenriched crop was enriched in 20 mL of RV broth and incubated at 42 °C for 24h. Also liver-spleen and ceca were enriched in 20 mL of RV broth and incubated at 42 °C for 24 h. Then 10 µL of the enriched crop, liver-spleen and ceca broth were streaked onto XLT4 agar (containing 25 µL and 20 µL of NO and NA respectively).

Furthermore, the concentration of *Salmonella* in the gastrointestinal (GI) tract was determined by diluting 0.25g of the cecal content in 2.25 mL of PBS, homogenized and 1 mL of the tenfold dilution of the cecal content was used to prepare serial dilution in 9 mL of PBS. And 0.1 mL of the serially diluted cecal content sample was spread on XLT4 agar (containing 25 µg and 20 µg of NO and NA respectively).
All the XLT4 agar sample plates were incubated at 37 °C for 24 h and the typical colonies of *Salmonella* morphology on XLT4 agar plates were enumerated and observed for the level of the pathogen in the GI tract and prevalence in the organs respectively.

Statistical analyses

The difference in the concentration of *Salmonella* (log_{10} CFU/g) infection in the cecal of the seeder birds, contact birds, motor activities, blood total leukocyte cell concentration and blood heterophil/ lymphocyte ratio concentration between birds reared under light intensity of 5 lux and 50 lux were compared for Analysis of variance (ANOVA) using PROC GLM procedure of SAS version 9.4 (SAS Institute, Cary, NC). None of the data sets was transformed, the data were analyzed for homogeneity of variance with Levene's test. The sample means were compared using DUNCAN MULTIPLE RANGE TEST of SAS version 9.4.

In addition, the difference between the prevalence of *Salmonella* in the crop, liver-spleen and ceca of the birds reared in the pens lit 5 lux and 50 lux were compared with either Fisher's Exact Test or Chi Square using PROC FREQ procedure of SAS version 9.4.

Results and Discussion

Horizontal transmission of *Salmonella* is one of the mechanisms of the infection of birds with this pathogen. Among the environmental factors that act as the carrier of this pathogen in the poultry house, *Salmonella* infected birds may be one of the agents of *Salmonella* dispersion that is difficult to control. This is because pest control, cleaning and disinfection of poultry pens prior to stocking may reduce the prevalence of pathogens in pens. But most birds that are infected with non-avian specific *Salmonella* serotypes are asymptomatic carrier of the pathogen (Cason, et al., 1994; Guard-Petter, 2001), hence the introduction of the pathogen to the pens and subsequently to the flock of birds may go unnoticed. *Salmonella* infection of chicks prior to brooding may occur due to the contamination of the eggs before or after oviposition (Gantois, et al., 2009; Gast, et al., 2004; Guard-Petter, 2001). The transmission of the pathogen may also occur at the hatchery (Cason, et al., 1994) and different serotypes of *Salmonella* have been isolated either from hatchery environment or transports pads (Bailey, et al., 2001; Byrd, et al., 1999). Strict biosecurity procedures on the farm as a *Salmonella* intervention strategy can reduce the prevalence of this pathogen in poultry houses and in birds.

The results of the prevalence of *Salmonella* in the ceca, liver-spleen and the crop of the seeder birds that were orally challenged with 7.7 $\log_{10}$ CFU/ mL of *Salmonella* Typhimurium at 3 d of age are presented in Table 5. There was no significant difference in the prevalence of the pathogen in any of the testes organs (crop, liver-spleen and ceca) of the birds irrespective of the lighting intensity in pens during rearing period.

Table 5. The prevalence of *Salmonella* in the organs of seeder birds reared under either 5 or 50 lux between 3 to 20 d of age

Light intensity (Lux)	*Salmonella* positive Crop/ Total Crop (%)	*Salmonella* positive Liver-spleen/ Total Liver-spleen (%)	*Salmonella* positive Ceca/ Total Ceca (%)	*Salmonella* positive Birds/ Total Birds (%)
5	0/ 10^a (0)	0/ 10^a (0)	6/10^a (60)	6/ 10^a (60)
50	0/ 10^a (0)	0/ 10^a (0)	5/ 10^a (50)	5/ 10^a (50)

Numbers with the same superscript letter a - b across the column are not significantly different P > 0.05.

Table 6 indicates the results of the *Salmonella* prevalence test on the organs of the contact birds. These results also indicated that there was no significant difference (P > 0.05) between the prevalence of *Salmonella* in the crop, liver-spleen and the ceca of the contact birds reared either under the light intensity of 5 or 50 lux.

Even though, there was no significant difference (P > 0.05) between the concentration of *Salmonella* cecal contents of the seeder birds reared under 5 and 50 lux (Table 7), there was a significant (P = 0.019) difference in the *Salmonella* cecal contents of the contact birds reared in the rooms lit with different light intensity (Table 7). The *Salmonella* in the cecal content of contact birds reared under 50 lux (0.84 $\log_{10}$ CFU/ g) was significantly higher than the *Salmonella* cecal contents of the contact birds reared under 5 lux (0.34 $\log_{10}$ CFU/ g).

Table 6. The prevalence of *Salmonella* in the organs of contact birds reared under either 5 or 50 lux between 3 to 20 d of age

Light intensity (Lux)	*Salmonella* positive Crop/ Total Crop (%)	*Salmonella* positive Liver-spleen/ Total Liver-spleen (%)	*Salmonella* positive Ceca/ Total Ceca (%)	*Salmonella* positive Birds/ Total Birds (%)
5	2/ 40[a] (5)	0 / 40[a] (0)	4/ 40[a] (10)	6/ 40[a] (15)
50	3/ 40[a] (7.5)	2/ 40[a] (5)	8/ 40[a] (20)	13/ 40[a] (32.5)

Numbers with the same superscript letter a - b across the column are not significantly different $P > 0.05$.

The difference in the concentration of *Salmonella* in the cecal content of the contact birds may depend on numerous factors, including the litter contrast. At higher light intensity, the rate of litter pecking by birds may be higher in comparison to the birds reared under lower intensities (Martin, 1989). Studies have indicated an increase in the motor activities of birds at higher light intensities (Blatchford, et al., 2012; Blatchford, et al., 2009; Newberry, et al., 1988). In this study, there was no significant difference ($P > 0.05$) between the motor activities of the birds reared in the pens lit with 5 lux (0.51 per daily photophase) and 50 lux (0.67 per daily photophase). The results of this study were inconsistent with other studies that indicated that motor activities of birds increased with increased light intensity.

Factors such as age of the birds when the motor activities were monitored, number of birds per pen and the duration of the motor activities in this study were different from

Table 7. Concentration of *Salmonella* in the ceca content of seeder and contact birds reared under either 5 or 50 lux between 3 to 20 d of age

Birds	*Salmonella* (log_{10} CFU/ g) in the cecal content of birds reared under 5 lux	*Salmonella* (log_{10} CFU/ g) in the cecal content of birds reared under 50 lux
Seeder	1.60 ± 0.46^{a}	1.54 ± 0.56^{a}
Contact	0.34 ± 0.08^{b}	0.84 ± 0.19^{a}

Numbers with the same superscript letter a - b across the column are not significantly different $P > 0.05$.

those of the other studies. All these factors may have caused the disparity in the result of motor activities observed in this study in comparison to other studies. For example, the age of the birds when the motor activities measurement was taken in this study was between 10 to 20 d, whereas in the Blatchford et al. (2009; 2012) the motor activities of the birds were measured from 3 – 6 wk of age. Study have suggested that the behavioral activities of birds such as feeding, drinking, walking, standing and other activities were significantly affected by the age of the birds that were monitored (Newberry, et al., 1988). In this study, the motor activities analyzed were monitored during the entire photophase, but in the other studies motor activities were monitored over a different time period. In the Blatchford et al. (2012), the data for the behavioral activities of the bird analyzed were the activities of the birds monitored in 48 h/ wk of the study. In addition, the number of the birds per pen in this study was lower than in some of the other studies that measured the relationship between light intensities and the behavior of birds. In Blatchford, et al (2009), the stocking density of the birds was 7.7 bird/ m2, whereas in

this study the stocking density was 5.81 birds/ m2, which is more consistent with current practices in the industry.

The analysis of the heterophil-lymphocyte ratio is used as one of the indicators of physiological stress in birds (Gross and Siegel, 1983). The difference in the intensity of light used in rearing birds in this study did not affect the blood heterophil/ lymphocyte ratio concentration between birds reared either under light intensity of 5 or 50 lux (Table 8). The heterophil-lymphocyte ratios of birds measured on 10 and 20 d of the study were not significantly different ($P > 0.05$). The means of heterophil-lymphocyte ratio of 10 d old birds reared under light intensity of 5 and 50 lux were 0.220 and 0.266 respectively with $P = 0.388$. While the means of the heterophil-lymphocyte ratio of 20 d of age birds reared with light intensity of 5 and 5 lux were 0.244 and 0.212 respectively with $P = 0.698$. Birds that are chronically stressed have been shown to be immunosuppressed and therefore will respond poorly to antigens (Freeman, et al., 1981; Kirby and Froman, 1991). Despite the differences in the activities of the birds reared under 5, 50 and 200 lux, there was no significant difference in the immunological response of the birds to various antigens (Blatchford, et al., 2009). The result of the physiological stress status of birds in this study is consistent with the Blatchford et al, (2009) who reported that there was no difference in the immune status of birds reared under different photophase light intensities. Source of lighting in poultry production might have impact on the indicators (heterophil/ lymphocyte ratio, total plasma corticosterone concentration and physical composite asymmetry) of stress in birds (Huth and Archer, 2015). Some LED bulb may

reduce the level of stress experienced by birds when compared to CFL even if the same light intensity is emitted by these bulbs (Huth and Archer, 2015).

Table 8. Heterophil/lymphocyte ratio content of blood from birds reared under either 5 or 50 lux between 3 to 20 d of age

Day	Heterophil-lymphocyte ratio in birds reared under light intensity of 5 lux	Heterophil-lymphocyte ratio in birds reared under light intensity of 50 lux
10	0.220 ± 0.29^{a}	0.266 ± 0.044^{a}
20	0.254 ± 0.072^{a}	0.212 ± 0.045^{a}

Numbers with the same superscript letter a – b across the row are not significantly different when P >0.05.

Leukocyte cells are the blood component that responds to any foreign organisms in the host. There was no significant difference in total leukocyte contents of the blood of the birds at both 10 and 20 d of age, irrespective of the photophase light intensities in the pens ($P > 0.05$). The actual concentration of the total leukocyte cell per 1 mL of blood sample was also determined, the detail of the total leukocyte cell is shown in Table 9. Both the innate and the adopted leukocyte cells are elicited in the presence of organisms through cytokine and chemokine responses (Ferro, et al., 2004; Hughes, et al., 2007; Shini, et al., 2010; Withanage, et al., 2005). Studies have shown that both the total leukocyte cells and its profile are adversely affected in the physiologically stressed animals (Dhabhar, 2002; Dhabhar, et al., 1994).

Table 9. Total leukocyte cell content of blood from birds reared under either 5 or 50 lux between 3 to 20 d of age

Day	Total leukocyte cell/ mL of blood of the birds reared under light intensities 5 lux	Total leukocyte cell/ mL of blood of the birds reared under light intensities 50 lux
10	75.50 ± 13.175^{a} (7.63×10^{6})	68.90 ± 9.514^{a} (6.96×10^{6})
20	70.20 ± 13.47^{a} (7.09×10^{6})	88.10 ± 15.80^{a} (8.90×10^{6})

Number with the same superscript letter a – b across the row are not significantly different when $P > 0.05$.

Conclusion

The differences in the photophase lighting intensities of 5 lux and 50 lux used in this study did not affect the prevalence of *Salmonella* in the birds. The concentration of *Salmonella* in the ceca of the birds reared under photophase light intensity of 50 lux was higher than in the birds reared under 5 lux, suggesting that rearing birds under low light intensities can reduce the concentration of *Salmonella* introduced to chicken processing plants during harvest. Rearing of birds under low light intensities of 5 lux is recommended in poultry production for the control of *Salmonella* since this light management practices can lower the concentration of the cecal *Salmonella* in birds. Hence the lighting management (5 lux) can reduce quantity of *Salmonella* introduced to the processing plant, thereby decreasing the risk of poultry product contamination with *Salmonella* while increasing the safety of the poultry supply chain. The motor activities,

the heterophil-lymphocyte ratio and the total leukocyte cells of the birds reared under photophase light intensity of either 5 or 50 lux from 3 to 20 d of age were the same. This implies that rearing birds between lighting intensities of 5 to 50 lux during photoperiod will not adversely affect the health and the welfare of the birds.

CHAPTER V

THE EFFECT OF LIGHTING SCHEDULE ON THE SHEDDING AND SPREADING OF *SALMONELLA* AMONG BROILER CHICKS HOUSED IN THE SAME PEN

Introduction

Intensive poultry production requires provision of a lighting system that meets the physiological requirement of birds throughout the preharvest period. Lighting systems in poultry production could be manipulated to increase productivity and reduce the cost of electricity (Appleby, et al., 1992; Buyse, et al., 1996; Rahimi, et al., 2005). Manipulation of the parameters of lighting may affect the behavior (Simmons, 1982), health (Classen, et al., 1991; Freeman, et al., 1981; Kirby and Froman, 1991; Lauber, 1991; Li, et al., 1995; Simmons, 1982; Wilson and Cunningham, 1980) and production performance of birds. The amount of dust generated in poultry houses may also be affected by the lighting management under which birds are reared (Al-Homidan, 2004; Calvet, et al., 2009; Ellen, et al., 2000).

Salmonella is one of the most reported causative agents of gastro enteritis all over the world. *Salmonella* is also the one of the most frequently isolated bacteria associated with human foodborne infection (Centers for Disease Control and Prevention, 2015; Liljebjelke, et al., 2005; Scallan, et al., 2011) in the United States and most developed nations (Baird-Parker, 1990). While different food commodities have been associated

with human foodborne *Salmonella* infection, poultry and poultry products are one of the food commodities that are mostly implicated in the incidence of human foodborne *Salmonella* infection outbreaks (Braden, 2006; Bryan, 1980; Centers for Disease Control and Prevention, 2015; Persson and Jendteg, 1992).

Several *Salmonella* serotypes have been isolated from either infected or contaminated poultry commodities at different stages of production (Bailey, et al., 2001; Bryan and Doyle, 1995; Byrd, et al., 1999; Liljebjelke, et al., 2005). Similarly, different *Salmonella* serotypes have also been indicted in human poultry borne *Salmonella* infection (Centers for Disease Control and Prevention, 2015). This statistic suggests that poultry commodities are carriers of some of the *Salmonella* serovar that are of significant health concern to humans.

Available information in the literature had suggested that an effective *Salmonella* control strategy must comprise *Salmonella* reduction, elimination and prevention of live bird infection with the pathogen. The need for preharvest *Salmonella* control intervention is supported by the fact that some of the *Salmonella* serovar that are isolated in the poultry and its environment were indistinguishable from the *Salmonella* strains isolated from poultry carcasses (Liljebjelke, et al., 2005; Rigby, et al., 1982).

Poultry may be infected with *Salmonella* through direct or indirect contact with the environmental agents. *Salmonella* has been isolated from litter/ chick tray liners (Byrd, et al., 1999; Davies and Breslin, 2003a; Kinde, et al., 2005), feed (Jones, et al., 1991; Jones and Richardson, 2004; Marin, et al., 2011), drinking water (Nayak, et al., 2003; Wray, et al., 1999; Yhiler and Bassey, 2015), air (Cason, et al., 1994; Gast, et al., 1998;

Kallapura, et al., 2014a; Kallapura, et al., 2014b), insects (Holt, et al., 2007; Kopanic, et al., 1994; Nakao, et al., 2015; Olsen and Hammack, 2000), rodents (Carrique-Mas, et al., 2009; Lapuz, et al., 2008) and caretakers (Marin, et al., 2011; Yhiler and Bassey, 2015).

In controlling *Salmonella* infection in poultry, strategies that reduced the prevalence of the pathogen in poultry houses have been introduced. Sanitation and disinfection of poultry houses after each growing and laying cycle is critical to controlling the pathogen in poultry environments. Pest control is considered to be one of the integral parts of poultry *Salmonella* control intervention and it has been implemented in many farms in controlling the spread of the pathogen. Furthermore, the adherence to the rules of hygiene by the farm workers cannot be overemphasized. Workers training and retraining on human role in the spread of the pathogen may play a significant role in the control of poultry infection with *Salmonella*.

Other strategies that have been implemented to increase the resistance of chicken to *Salmonella* infection include vaccination (Cogan and Humphrey, 2003; Smith, 1956; Zhang-Barber, et al., 1999). Control approaches include feed and water additives, antibiotics reduced fecal shedding of *Salmonella* (Evangelisti, et al., 1975; Girard, et al., 1976) and probiotics increased resistance of poultry to *Salmonella* infection (Higgins, et al., 2007; Hosoi, et al., 2000; Line, et al., 1998). Application of the Nurmi concept in young chicks is also a reliable approach to increasing *Salmonella* resistance in birds (Nuotio, et al., 1992; Nurmi, et al., 1992; Nurmi and Rantala, 1973). Studies on an experimental chlorate product have also indicated the efficacies of the product in

reducing *Salmonella* prevalence in market age birds especially during the feed withdrawal period (Byrd, et al., 2003; Byrd, et al., 2008).

While some successes of these *Salmonella* control strategies in poultry have been reported, more interventions are still needed. Management practices may increase poultry exposure or susceptibility to environmental contaminants such as *Salmonella*. For example, the motor activities, immune status, physiological stress and dust generation in poultry may be impacted by the lighting management systems. These impacts of lighting may adversely affect *Salmonella* infection in poultry. It may be advantageous that the poultry industry designs and implements a lighting system as a strategy to control *Salmonella* infection in birds.

The role of lighting on *Salmonella* infection in poultry was demonstrated in a report on a multistate survey of the effect of different lighting programs in commercial poultry farms on the prevalence of *Salmonella* on carcasses after harvest. The study suggested that broilers reared under the long period of reduced lighting programs had lower prevalence of *Salmonella* on their carcasses (Volkova, et al., 2010). The length of photoperiod may increase the episode of physiological stress in birds (Campo, et al., 2007) . And this may lower the immune response of the birds to pathogenic infection (Freeman, et al., 1981; Kirby and Froman, 1991). Dust is a carrier of *Salmonella*, and more dust is generated during photophase (Al-Homidan, 2004). This implies that the dispersion of *Salmonella* within the poultry house may increase with the length of photoperiod.

Therefore, a management technique that will optimally manipulate the daily photoperiod in poultry might reduce the factors that increase the susceptibility of poultry and the exposure to *Salmonella* infection. Hence the overall goal of this study was to evaluate the effect of two lighting program schedule practices in commercial poultry farms and their effects on *Salmonella* infection in poultry.

The objectives of the research include:

- To investigate the effect of intermittent and continuous lighting scheme on the prevalence and concentration of *Salmonella* in birds of the same pen
- To evaluate the differences in the effect of the lighting schemes on blood heterophil-lymphocyte ratio (indicator of stress) of birds
- To determine the effect of lighting scheme on the concentration of total leukocyte cells in the blood of the birds
- To establish a relationship between the heterophil-lymphocyte ratio, total leukocyte cells and *Salmonella* infection status of the birds

Materials and methods

Pen preparation, grouping of birds and blood sampling

In two replications, a total of 100- commercial d old Ross 708 broiler chicks of the same flock were obtained from a local commercial hatchery and were transferred to the Southern Plains Agricultural Research. Prior to the start of the study, all the birds were

screened for the presence of *Salmonella* by retrieving and analyzing the paper pad tray liner that was used to transport all the broiler chicks from the hatchery. The birds that were *Salmonella* negative were divided into four groups with 25 birds per pen and two pens per room. Birds in each room were designated as either control or treatment birds. All the birds in same room were reared under the same conditions throughout the study. The birds were fed to starter crumble feed diet according to the standard of the industry (Leeson and Summers, 2005) until 14 d of age, and standard grower diet up to 20 d of age when the study ended. The birds were treated according to the guideline recommended by the United States Department of Agriculture (USDA) Animal Care and Use Committee. The ambient temperature and the relative humidity in the pens were maintained according to the breeder recommendation (Ross PS Management Handbook) throughout the study.

The dimension of each of the pens was 4.301 m^2. On day 10 of the study, blood samples were collected from the jugular vein of five birds from each of the pens. Each blood sample was transferred into Vacutainer EDTA 10 mL PK100 (Zarnitsyna and Zhu, 2011) and was used for both leukocyte and heterophil-lymphocyte ratio analyses. All the blood samples were held at ambient temperature for about 3 h prior to being analyzed. On completion of blood sampling procedures, all the birds were returned to their respective pen and lighting program schedules commenced. The lighting program continued for another 10 d, after which a second blood sampling (day 20) was conducted on same birds that were previously sampled for blood. On completion of the blood sampling, all the birds were euthanized by carbon dioxide (CO_2), which was verified by

cervical dislocation as approved in the American Veterinary Medical Association (AVMA) guidelines on euthanasia (American Veterinary Medical Association, 2007). The cadavers were disinfected and necropsied for *Salmonella* analysis.

Preparation of *Salmonell*a culture and oral challenge of birds with the pathogen

Salmonella Typhimurium was used to orally challenge birds in this study; this was because the serovar (*Salmonella* Typhimurium) is one of the most reported causes of human salmonellosis in the United States (FoodNet, 2010). *Salmonella* Typhimurium is also one of the most frequently isolated *Salmonella* serotype in young chicken (Food Safety and Inspection Services, 2009). A pure culture of Novobiocin (NO, Sigma-Aldrich, St. Louis, MO) and Nalidixic Acid (NA, Sigma-Aldrich, St. Louis, MO) resistant *Salmonella* Typhimurium was retrieved from -80 C freezer, thawed and 10 µL of the pathogen was transferred into 10 mL of Tryptic Soy Broth (TSB, Becton, Dickson and Company, Franklin, NJ) that contained 25 µg and 20 µg of NO and NA respectively. The culture preparation was completed as described in Byrd et al (2003:2008). And the absorbance of the suspension of the NO and NA resistant *Salmonella* Typhimurium was measured using spectrophotometer (Spectronic 2OD by Milton Roy Company, Ivyland, PA) at wavelength of 625 nm. The absorbance level of this suspension was adjusted by adding more PBS to the suspension of the pathogen until the absorbance level was equal to 1.58 (10^8 CFU/ mL of NO and NA resistant *Salmonella* Typhimurium).

On 3 d of the study, five birds (seeder birds) from each of the groups were randomly selected, wing banded, labelled with spray paint and orally challenged with 8.02 $\log_{10}$

CFU/ mL of *Salmonella* Typhimurium. Meanwhile, the remaining twenty birds (contact birds) in each of the pens remained unchallenged.

Lighting schedule

Throughout the study, feed and water were provided *ad libitum* and the photoperiod of the bird was controlled with the adjustment of the photophase/scotophase (photophase = lighting (L) period, scotophase = darkness (D) period). During photophase, light source was fluorescent bulb with intensity that ranged from 145 to 175 lux depending on the part of the room. The daily period of light in all the pens was the same (16 h). However, the scheduling of the light/darkness for the two groups was varied; the lighting regimen in the pens housing birds in groups A1 and A2 (continuous lighting) was 16L:8D, while the light scheduling in the pens housing birds in the groups B1 and B2 (intermittent lighting) was adjusted to 4L:2D:4L:2D:4L:2D:4L:2D. This lighting program in all the pens was introduced on 10 d and continued until the completion of the study (20 d). In summary, the total 16 h of daily photoperiod was applicable to all the 4 pens.

Preparation and analyses of blood samples for leukocyte, and heterophil/ lymphocyte ratio

Leukocyte and heterophil-lymphocyte ratio of the blood samples were determined using the procedure described in Natt and Herrick (1952) and Genovese et al. (1998) respectively. Briefly, the total leukocyte content of each blood sample was determined

by transferring 10 µL of blood sample from the Vacutainer EDTA 10 mL PK100 (Zarnitsyna and Zhu, 2011) to a sterile 2 mL vial that contained 1000 µL of Natt and Herrick diluent. The blood- Natt and Herrick diluent mixture was homogenized, 15 µL of the blood-diluent mixture was transferred onto hemocytometer and the total leukocyte cell content of each blood sample was read under a light microscope. The heterophil-lymphocyte ratio, an indicator of stress (Gross and Siegel, 1983) was determined to evaluate the effect of the different lighting schemes (either continuous or intermittent lighting) on the birds. And the cell ratio was analyzed by making blood smear on slide and subsequent staining with Hema 3-stained cytospin (Shandon Scientific, Pittsburgh, PA) (Genovese, et al., 1998). In summary the blood smears were stained in 3 step stain reagents by 1 min immersion each in fixative, solution I and finally in solution II. Stained blood smears were rinsed in distilled water, dried, both heterophil and lymphocyte cells were read under light microscope and the ratio of the heterophil to lymphocyte per blood smear was calculated.

Microbiological analyses

Screening of day old chicks for* Salmonella *infection

The tray liner was preenriched (Waltman and Gast, 2008) by aseptically transferring it into a sterile bag that contained 200 mL of Buffer Peptone Water (BPW, Becton, Dickson and Company, Franklin, NJ), hand massaged for 1 min, and was incubated at 37 °C for 24 h. Then 0.2 mL of the preenriched tray pad BPW was transferred into 20 mL of Rapport Vasiliadis (RV, Becton, Dickson and Company, Franklin, NJ) Broth, and was

incubated at 42 °C for 24 h. Then a loopful (10 µL) of the enriched sample was streaked in triplicate onto Xylose-Lysine-Tergitol 4 agar (XLT4, Hardy Diagnostic, Santa Maria, CA), incubated at 37 °C for 24 h and observed for typical *Salmonella* colony.

Determination of* Salmonella *infection in birds

Crop, liver-spleen and ceca of each of the birds were retrieved on the 20 d of the study. The concentration of the *Salmonella* in the ceca was determined by transferring 0.25 g of cecal content into 2.25 mL of PBS and 1 ml of the diluted cecal content was used to prepare a serial dilution. And 0.1 mL of the serially diluted cecal content was plated on Xylose-Lysine-Tergitol 4 (XLT4) agar (containing 25 µL and 20 µL of NO, NA respectively). All the plates were incubated at 37 °C for 24 h, and the typical colony of *Salmonella* morphology was enumerated.

For the prevalence of *Salmonella* in each bird, crop of each of the birds was preenriched in 55 mL of BPW and incubated at 37 °C for 24 h. Each preenriched crop was enriched in 20 mL of RV broth. Also each liver-spleen and cecum was enriched in 20 mL of RV broth and all the samples were incubated at 42 °C for 24 h. And 10 µL of each of the enriched crop, liver-spleen and cecum was streaked onto XLT4 agar. All the XLT4 agar plates were incubated at 37 °C for 24 h. The typical colony of *Salmonella* morphology on XLT4 agar was observed.

Statistical analyses

Prevalence and the cecal content concentration (log_{10} CFU/ g) of *Salmonella* between seeder birds reared under different lighting scheme were compared. Also the prevalence and the concentration of *Salmonella* infection in contact birds between both groups were separately compared. The Analysis of variance (ANOVA) was used to compare difference in the concentration of the *Salmonella* (log_{10} CFU/ g) in the cecal content of the birds, the total leukocyte cell and heterophil-lymphocyte ratio between birds in the intermittent light and continuous light groups using PROC GLM procedure of SAS version 9.4 (SAS Institute, Cary, NC). The data sets were tested for homogeneity of variance (Levene's Test). The mean separation between both groups of bird was analyzed using Duncan Multiple Range Test.

The difference in the prevalence *Salmonella* in the organs (crop, liver-spleen and ceca) of seeder birds between both groups of birds was compared using Fisher's Exact Test with PROC FREQ procedure of SAS version 9.4. While the difference in the prevalence of the pathogen in the organs of the contact birds between both lighting groups was compared using Chi Square test with PROC FREQ procedure of SAS version 9.4. The significant difference between the groups was when $P < 0.05$.

Results and discussion

Salmonella infection status of birds

The culture of the tray liner confirmed that all the birds used for this study were *Salmonella* negative prior to the start of the experiment.

There was no significance difference ($P > 0.05$) in the prevalence of *Salmonella* in the crop, liver-spleen and ceca of the seeder birds reared either under continuous lighting or intermittent lighting schedule (Table 10). Furthermore, there was no significance difference ($P > 0.05$) between the concentration of *Salmonella* in the ceca of seeder birds in both continuous lighting (4.12 $\log_{10}$ CFU/ g of cecal content) and intermittent lighting (4.27 $\log_{10}$ CFU/ g of cecal content). And the $P = 0.831$.

There was also no significant difference in the prevalence of *Salmonella* between the crop and liver-spleen of the contact birds reared in continuous and intermittent lighting schedule (Table 11). However, there was a significant difference ($P = 0.0002$) between the prevalence of *Salmonella* in the ceca of the contact birds. Contact birds reared under continuous had higher prevalence of *Salmonella* in the cecal (100 %) than the contact birds in the intermittent lighting scheme (70 %).

Furthermore, the concentration of *Salmonella* in the cecal content of the contact birds reared under continuous lighting (4.91 $\log_{10}$ CFU/ g) was significantly higher than those of the contact birds in the intermittent lighting (3.33 $\log_{10}$ CFU/ g) group ($P < 0.0001$).

This study supports other studies which suggested that few *Salmonella* infected birds

Table 10. The prevalence and concentration of *Salmonella* in the organs and cecal content of the seeder birds reared under either continuous lighting or intermittent lighting schedule

Lighting schedule	*Salmonella* positive crop/total crop (%)	*Salmonella* positive liver-spleen/total liver-spleen (%)	*Salmonella* positive ceca/total ceca (%)	*Salmonella* (log_{10} CFU/ g of cecal content)
Continuous	4/10 (40)[a]	4/10 (40)[a]	10/10 (100)[a]	4.12 ± 0.588[a]
Intermittent	3/10 (30)[a]	5/10 (50)[a]	9/10 (90)[a]	4.27 ± 0.363[a]

Numbers with the same superscript letter a - b across the column are not significantly different P > 0.05.

in a poultry flock might spread the pathogen to the entire flock (Snoeyenbos, et al., 1969). This study indicated that there was an increase in the number of the birds infected with *Salmonella* at the end of the study in comparison to the start of the experiment when none of the contact bird was positive to the pathogen irrespective of the lighting schedule. The result of the study also suggested that there was a reduction in the prevalence and concentration of the pathogen in the ceca of the contact birds reared under intermittent lighting program. This decrease may be because birds under intermittent lighting program had lesser opportunity to exhibit foraging behavior. Unlike the birds that were reared in the continuous lighting pen that may have exhibited more foraging behaviors such as litter pecking. Reports have suggested that birds reared under intermittent lighting program feed more on diet, whereas those reared under continuous lighting tend to nibble more on diet (Lewis and Morris, 2006).

Table 11. The prevalence and concentration of *Salmonella* in the organs and cecal content of the contact birds reared under either continuous lighting or intermittent lighting schedule

Lighting schedule	*Salmonella* positive crop/total crop (%)	*Salmonella* positive liver-spleen/total liver-spleen (%)	*Salmonella* positive ceca/total ceca (%)	*Salmonella* (log_{10} CFU/ g of cecal content)
Continuous	19/40 (47.5)[a]	19/40 (47.5)[a]	40/40 (100)[a]	4.91 ± 0.106[a]
Intermittent	13/40 (32.5)[a]	21/40 (52.5)[a]	28/40 (70)[b]	3.33 ± 0.283[b]

Number with the same superscript letter a - b across the column are not significantly different when P > 0.05.

Fecal shedding of *Salmonella* by the seeder might be the agent of dispersal of the pathogen to the litter and the contact birds may have been exposed to this pathogen through litter pecking. Intermittent scotophase and photophase may have reduced the extent of litter pecking, since the birds will spend more of the photophase to feed (Lewis and Morris, 2006). Therefore, the differences in the foraging behavior of birds under both lighting program in this study may be the one of the factors responsible for the differences in the *Salmonella* content of the ceca between both groups of birds.

Another mechanism that may have contributed to the higher concentration and prevalence of *Salmonella* in the ceca of the birds reared under continuous lighting might be the long hour of scotophase. During scotophase period, foraging activities might be very low, during 8 h of scotophase might be similar to 8 h of feed withdrawal practice

prior to harvesting. Studies have indicated that during the period of feed withdrawal in chicken, there was an increase in the crop pH (Corrier, et al., 1999; Humphrey, et al., 1993). Feed withdrawal in chicken has also been associated with an increase in the population and virulence of *Salmonella* (Durant, et al., 1999; Ramirez, et al., 1997).

In this study, the higher prevalence and concentration of *Salmonella* observed in the ceca of the contact birds reared under continuous lighting scheme might be due to the long hour of scotophase.

Blood analyses

There was no significance difference in the heterophil-lymphocyte ratio between both groups of birds on at both 10 and 20 d of age, with P of 0.82 and 0.122 respectively. Furthermore, at 10 and 20 d of age, the heterophil-lymphocyte ratio of the birds reared under continuous light was 0.193 and 0.442 respectively. Whereas the heterophil-lymphocyte ratio of the birds reared under intermittent lighting was at both days (10 and 20) was 0.184 and 0.270 respectively. And the ratio of the heterophil/lymphocyte cell content of both groups of birds on 10 and 20 d of the study is shown in the Table 12.

However, there was a significance difference (P = 0.008) in the heterophil-lymphocyte ratio between the time before (10 d) and after (20 d) introduction of the lighting scheme in the birds reared under continuous lighting program. Unlike in the birds reared under intermittent lighting. This indicated that the continuous lighting

Table 12. Blood heterophil/lymphocyte ratio content of blood on both 10 and 20 d of the study of seeder birds reared under either continuous lighting or intermittent lighting schedule

Lighting schedule	10 d (Heterophil/ Lymphocyte ratio)	20 d (Heterophil/ Lymphocyte ratio)
Continuous	0.193 ± 0.027^{a}	0.442 ± 0.079^{a}
Intermittent	0.184 ± 0.029^{a}	0.270 ± 0.068^{a}

Number with the same superscript letter a - b across the column are not significantly different when $P > 0.05$.

scheme induced some degree of stress on the birds between 10 and 20 d, whereas the intermittent lighting scheme did not induced stress on the birds.

The change in the heterophil lymphocyte ratio of blood might be due to the chronical increase in the level of stress induced hormone in the blood plasma. Animal responds to chronic stress by production and secretion of corticosterone (Selye, 1936). The increase level of corticosterone in the circulating plasma will result to the condition known as lymphocytopenia, which is the reduction in the circulating lymphocyte due to the depression of lymphoid tissues (Quinteiro-Filho, et al., 2010; Smith, 2003). Animal undergoing physiological stress might be more susceptible to infection due to lower immune response (Freeman, et al., 1981; Kirby and Froman, 1991). Therefore, the higher prevalence and concentration of cecal *Salmonella* infection in the birds reared under continuous lighting scheme might also be alluded to the higher physiological stress in this group of birds.

Stress hormone such as catecholamine (Cannon, et al., 1929), have been shown to enhance growth and expression of virulence factors in gram negative bacteria (Belay and

Sonnenfeld, 2002; Lyte and Ernst, 1992; Rahman, et al., 2000). Although, birds in the continuous lighting group were not stressed in comparison to the birds reared in intermittent lighting schedule, the higher level of epinephrine and norepinephrine might also explain why the *Salmonella* infection in the ceca was significantly different in them. This study is consistent with the finding in Rahman, et al. (2000) that indicated an increase in the growth *Salmonella* Typhimurium in the presence of stress hormone.

Leukocyte cells are the blood components that are responsible for defending host against invading microorganisms. In this study, there was no significant difference in the leukocyte cell between both groups of birds on 10 and 20 d, and the P was 0.446 and 0.317 respectively. The detail of the leukocyte cell concentration in the blood samples of the birds is indicated in Table 13.

Meanwhile, the circulating leukocyte cell decreased at 20 d in both groups of birds. This suggested that the lighting system had inhibitory effect on the total leukocyte cells. The reduction in the concentration of the leukocyte cells and increase in the heterophil/ lymphocyte ratio over time in this study might be caused by the source light. Fluorescent light may increase stress level in birds (Huth and Archer, 2015), the relative high concentration of heterophil/ lymphocyte ratio observed in this study may be due to the lighting source.

In this study, the reduction in the total leukocyte cell in the birds at 20 d of age might also be due to the *Salmonella* infection status of the birds, since large per cent of the birds samples were *Salmonella* positive. Reduction in both the innate and adapted immune cells in stressed animals had been reported (Dhabhar, et al., 1994; Gross and

Siegel, 1983; Gross and Siegel, 1985; Gross, et al., 1980; Mcfarlane and Curtis, 1989; Zorrilla, et al., 2001). Determination of the actual stressor that was responsible for this reduction in total leukocyte cell in the study may be an interesting research objective.

Table 13. Leukocyte content of blood on both 10 and 20 d of the study of seeder birds reared under either continuous lighting or intermittent lighting schedule

Lighting schedule	10 d Total Leukocyte cell/ mL of blood	20 d Total Leukocyte cell/ mL of blood
Continuous	101.8 ± 9.827^{a} (1.028×10^{7})	49.1 ± 4.584^{a} (4.959×10^{6})
Intermittent	90.9 ± 9.923^{a} (9.180×10^{6})	56.9 ± 6.125^{a} (5.748×10^{6})

Number with the same superscript letter a - b across the column are not significantly different when $P > 0.05$.

Conclusion

From this study, application of intermittent lighting scheme in broiler production, especially at young age may reduce *Salmonella* transmission among birds. From a food safety perspective, intermittent lighting is a preferable choice of poultry lighting over continuous lighting, because birds reared under intermittent lighting may be carriers of lower cecal concentration of *Salmonella* even if they are infected with the pathogen.

In addition, birds reared under the continuous lighting experienced physiological stress between 10 to 20 day of age, whereas the birds reared under intermittent lighting scheme were not stressed. This indicated that the welfare of the birds reared in continuous lighting may have been negatively impacted within 10 to 20 d in comparison

to birds reared under intermittent lighting. Therefore, intermittent lighting scheme is recommended to poultry farmers to improve on the welfare and health of birds, especially at early stages of life.

CHAPTER VI

EFFECTS OF TEMPERATURE ELEVATION ON THE HORIZONTAL TRANSMISSION OF *SALMONELLA* AMONG BROILER CHICKENS AT 4 WK OF AGE REARED IN THE SAME PEN

Introduction

In commercial chicken farming, one of the environmental factors that must be controlled is temperature. Poultry are homeotherms and an adequate ambient temperature in the pens is essential for the maintenance of the homeostasis condition. During thermogenesis, chicken can increase or decrease the rate of thermal loss to the surrounding medium depending on the prevailing environmental temperature (Osbaldiston and Sainsbury, 1963). Imbalance between the flow of heat energy between animals and the its surrounding medium has been described as heat stress (Lara and Rostagno, 2013). Heat stress may have negative impact on productivity performance of broilers (Donkoh, 1989; Howlider and Rose, 1989). Also the digestibility of feed may be adversely affected in the birds that are exposed to heat stress (Bonnet, et al., 1997; Larbier, et al., 1993). Like in all other animals, birds reared in an ambient temperature beyond their thermoneutral zones could be immunosuppressed. Immune response of heat stressed birds is usually poor when compared to the birds reared in an environment that did not imposed heat stress on them (Smith, 2003; Thaxton and Siegel, 1970; Zahraa and Ghamdi, 2008).

Studies have suggested a lower resistance of heat stressed birds to *Salmonella* infection, due to modulation of the intestinal microbiota of the birds and reduction in the competitive exclusion potential (Bailey, 1988; Burkholder, et al., 2008). Other environmental conditions could also induce stress response in chicken, and may have an effect on the susceptibility of chicken to *Salmonella* infection. For example, housing method (Gast, et al., 2014; Rigby and Pettit, 1979), stocking density (Holt, et al., 1998) and lighting system (Volkova, et al., 2010) may affect the prevalence of *Salmonella* in birds.

Some of the stress hormones such as norepinephrine that are produced and secreted in stressed animals (Cannon, et al., 1929) act as autoinducer for gram negative bacteria. The growth and virulence of this group of microorganisms increases in the presence of these hormones (Belay and Sonnenfeld, 2002; Lyte and Ernst, 1992). In the presence of norepinephrine at the concentration of 5×10^{-5} M/ mL, growth and production of enterotoxin by *Salmonella* Typhimurium increased in tenfold and eightfold respectively (Rahman, et al., 2000). An increase in the fecal shedding of *Salmonella* has been observed in stressed birds (Nakamura, et al., 1994a; Nakamura, et al., 1994b). Exposure of birds to environmental stressors might increase the prevalence of the pathogen in the poultry farms.

Numerous intervention approaches have been introduced to control *Salmonella* in poultry at the preharvest stage of production. Sanitation and biosecurity approach is applied in poultry houses to reduce the introduction of *Salmonella* to poultry flocks. Vaccination of poultry is also one of the approaches that have been effectively applied to

control *Salmonella* Enteritidis in laying hens and eggs (Cogan and Humphrey, 2003). Another strategy that has been employed to control *Salmonella* includes the use of feed additives. For example, reduction in the level and prevalence of *Salmonella* Typhimurium in young birds was significantly reduced in the group of birds that were fed with feed containing *Saccharomyces bourlardii* (Line, et al., 1998). Studies have also revealed that supplementing poultry feeds with antibiotics (Evangelisti, et al., 1975; Girard, et al., 1976), probiotics (Higgins, et al., 2007; Higgins, et al., 2010; Patterson and Burkholder, 2003), and prebiotics (Eeckhaut, et al., 2008; Spring, et al., 2000a) increased the resistance of the birds to *Salmonella* infection. Other feed or water additives that have also been shown to effectively lower the susceptibility of poultry to *Salmonella* infection include: synbiotics (Collins and Gibson, 1999; Nisbet, et al., 1993b), acidification with short chain fatty acid (Fernández-Rubio, et al., 2009; McHan and Shotts, 1992), experimental chlorate product (Byrd, et al., 2003; Byrd, et al., 2008) and others.

The association of live poultry with human *Salmonella* infection has been increasing, for example between the years 2010 to 2015, about 44 % of poultry related human *Salmonella* infection outbreaks were due to contact with live poultry (Centers for Disease Control and Prevention, 2015). The report also suggested that more *Salmonella* control interventions are still needed to reduce *Salmonella* infection in poultry. Furthermore, the environmental temperature of birds may affect the susceptibility of the birds to *Salmonella* infection and the fecal shedding of the pathogen (Thaxton, et al., 1971). An increase in the fecal shedding of *Salmonella* (Soerjadi, et al., 1979) and the

severity of *Salmonella* infection (Thaxton, et al., 1974) was observed in the cold stressed birds. Also the ex vivo study on the concentration of *Salmonella* in the ileal tissue of birds subjected to heat stress of 30 °C for 24 h prior to euthanasia indicated that there was a 0.27 $\log_{10}$ increase in the tissue of the heat stressed birds compared to the birds reared under 23 °C (Burkholder, et al., 2008).

Available information in the literature suggested an impact of management practices in the poultry husbandry on the fecal shedding, organ concentration and prevalence of *Salmonella* in poultry. There the goal of this study was to determine the effect of ambient temperature on the horizontal transmission of *Salmonella* in birds reared in the same pen. And the objectives of the study are as follows:

- To determine the effect of ambient temperature on the cecal concentration and the prevalence of *Salmonella* in birds reared in the same pen.
- To assess the effect of ambient temperature on the body weight of birds, the ratio of cecal tonsil and the adrenal gland of the birds to the body weight.
- To evaluate the impact of ambient temperature on the ratio of heterophil-lymphocyte contents in the blood of the birds.

Materials and methods

Pen temperature and experimental design

Total of 100 Ross 708 broiler chicks of day old of age were used for this study. The broiler chicks were obtained from a local commercial hatchery and transferred to the Southern Plains Agricultural Research Center. All the birds were taken care of according to the guidelines of Animal Care and Use Committee of the USDA. The broiler chicks were provided with poultry starter feed up to 14 d of life, and were provider with grower feed until 28 d of age. The ingredients and the proportion for feed mixing was in line with the standard feed formulation practice in the poultry industry (Leeson and Summers, 2005). Feed and water were provided *ad libitum*. The birds were reared on floor pens covered with new wood shaving of 2.5 cm height. And the pens were kept in dried state throughout the study.

The paper liners from the chick transport boxes were cultured successively in the buffered peptone water (BPW, Becton, Dickson and Company, Franklin, NJ), Rapport Vasiliadis (RV, Becton, Dickson and Company, Franklin, NJ) and on Xylose-Lysine-Tergitol 4 (XLT4, Hardy Diagnostic, Santa Maria, CA) agar plates as described previously and examined for *Salmonella* (Andrews, et al., 1978). *Salmonella* spp were not detected in the paper liner. Birds transported on *Salmonella* negative paper pad tray liners were divided into 4 groups, with each of the groups containing 25 birds. Two groups of the birds were reared in the same room but in different pens, while the other two groups of birds were reared in another room but in different pens as well. The temperature in both rooms housing the birds were adjusted to 35 °C for 1 wk and was

reduced to 32 °C, and this temperature was maintained until the birds were 2 wk of age. However, the ambient temperature in the room housing the birds in the control groups was further reduced by 3 °C per wk until the end of the study, thereby the ambient temperature in the two control pens was lowered to 26 °C by the start of week four of the study. Meanwhile, the temperature in the room housing the birds in the treatment groups was kept unchanged at 32 °C for the remaining period of the study. Constant ambient temperature of 32 ºC exerted heat stress on broiler chicken between 2 to 4 wk of age (Azad, et al., 2010; Geraert, et al., 1996). On 3 d of the study, all the birds were wing banded and 5 birds from each of the pens were challenged with 8.33 $\log_{10}$ CFU/ mL of pure culture of *Salmonella* Typhimurium and all the birds were returned to their respective pen. Furthermore, on both 10 and 25 d of the study, blood samples of all the birds were collected, transferred into vacutainer EDTA 10 mL PK100 to prevent clotting (Xie, et al., 2015) and were used for heterophil-lymphocyte ratio analysis. And on the 28 d, the last day of the study, all the birds were euthanized by inhalation of carbon dioxide ($CO_{2)}$ and was verified by cervical dislocation (Leary, et al., 2013). The cadavers were disinfected, necropsied, the crop, liver-spleen and ceca of each of the birds were aseptically retrieved for *Salmonella* analysis. Also the cecal tonsil and the adrenal gland were weighed and used for physiological stress analyses.

Preparation of the *Salmonella* Typhimurium culture

Salmonella Typhimurium was used to orally challenge birds in this study; this was because the serovar (*Salmonella* Typhimurium) is one of the most reported causes of

human foodborne salmonellosis in the United States (FoodNet, 2010). *Salmonella* Typhimurium is also one of the most frequently isolated *Salmonella* serotype in young chicken (Food Safety and Inspection Services, 2009). Pure culture of Novobiocin (NO, Sigma-Aldrich, St. Louis, MO) and Nalidixic Acid (NA, Sigma-Aldrich, St. Louis, MO) resistant *Salmonella* Typhimurium was prepared as described in the studies on experimental chlorate products (Byrd, et al., 2003; Byrd, et al., 2008). The absorbance of the suspension of the NO and NA resistant *Salmonella* Typhimurium was measured in spectrophotometer (Spectronic 2OD by Milton Roy Company, Ivyland, PA) at wavelength of 625 nm. The absorbance level of the pathogenic suspension was adjusted by adding more PBS to the suspension of the pathogen until the absorbance level was equal to 1.58 (10^8 CFU/ mL of NO and NA resistant *Salmonella* Typhimurium).

Physiological stress indicator analyses

The heterophil-lymphocyte ratio content of blood samples collected from all the birds at both 10 and 25 d of age was analyzed by making a smear of the blood sample on a slide, fixed and stained in Hema 3-stained cytospin (Shandon Scientific, Pittsburgh, PA) (Genovese, et al., 1998). Each of the cells (heterophil and lymphocyte) was enumerated under a light microscope and the ratio was calculated.

The body mass (Kg) of each of the live birds was determined at 28 d of age prior to euthanasia. The relative mass of each of the adrenal gland (Freeman, et al., 1981) and cecal tonsil (Quinteiro-Filho, et al., 2010) to the live birds' mass was measured immediately after necropsy.

Microbiological analyses

Prevalence of Salmonella *in day old chicks*

The paper pad tray liner was assayed for *Salmonella* as described in (Andrews, et al., 1978; Waltman and Gast, 2008). In summary, the tray line was preenriched BPW, enriched in RV broth and streaked onto XLT4 agar in triplicate. And the plates were observed for typical morphology of *Salmonella* colony.

Determination of ceca colonization by Salmonella

The 0.25g of cecal content was diluted in 2.25 mL of Phosphate Buffer Saline (PBS, Becton, Dickson and Company, Franklin, NJ), homogenized. And 1 mL of the cecum content homogenate was used to prepare a 10- fold serial dilution in PBS and 0.1 mL of the serially diluted samples was spread on XLT 4 agar (containing 25 µL and 20 µL of Novobiocin and Nalidixic Acid respectively). Meanwhile the crop was preenriched in BPW and incubated at 37 °C for 24 h. Each of the preenriched crop, liver-spleen and cecum was enriched in 20 mL of RV Broth, vortexed and incubated at 42 °C for 24 h. A 10 µL loopful of the enriched samples were streaked onto XLT4 agar. All the inoculated XLT4 agar plates were incubated at 37 °C for 24 h, and the typical colony of *Salmonella* morphology in the agar was observed and enumerated.

Statistical analyses

Concentration of cecal content *Salmonella* ($\log_{10}$ CFU/ g), the blood heterophil/ lymphocyte ratio, the relative weight of the adrenal gland, the relative weight of the ceca

tonsil and the live weight between the birds (seeder and contact) reared in a normal and elevated ambient temperature were compared using Analysis of variance (ANOVA). The ANOVA was determined using PROC GLM procedure of SAS version 9.4 (SAS Institute, Cary, NC). And the means difference between the samples from group bird were analyzed using Duncan Multiple Range Test, and there was significant difference when $P < 0.05$.

Difference in the prevalence *Salmonella* in the (organs) crop, liver-spleen and ceca of seeder birds between both groups of birds was compared using Fisher's Exact Test. The difference in the prevalence of the pathogen between the organs of contact birds was determined using Chi Square Test. Both Fisher's Exact Test and Chi Square Test calculated using PROC FREQ procedure of SAS version 9.4, significant difference between the groups was when $P < 0.05$.

Results and discussion

There was no significant different ($P > 0.05$) between the prevalence of *Salmonella* in the crop, liver-spleen and the ceca of the seeders birds reared under normal and elevated ambient temperatures from14 to 28 d (Table 14). There was also no significant difference between the concentrations of *Salmonella* in the cecal contents of the seeder birds reared under any of the two ambient temperatures.

Table 14. Prevalence of *Salmonella* in the organs and concentration (log_{10} CFU/ g) in the ceca of the seeder birds reared under either normal or elevated ambient temperature for their age

Group	Crop/ Total Crop (%)	Liver-spleen/ Total Liver-spleen (%)	Ceca/ Total Ceca (%)	Infected bird/ Total bird (%)	*Salmonella* (log_{10} CFU/ g)in the cecal content
Control	9/ 10 (90)[a]	7/ 10 (70)[a]	6/ 10 (60)[a]	9/ 10 (90)[a]	3.30 ± 0.56[a]
Treatment	6/ 8 (75)[a]	2/ 8 (25)[a]	6/ 8 (75)[a]	7/ 8 (87.5)[a]	4.17[a] ± 0.61[a]

Number same superscript letter a-b across the column are not significantly different.

Significant difference was when P value < 0.05.

Control (Ambient temperature was 32 °C from 7 to 14 d of age, 29 °C from 15 to 21 d of age and 26 °C from 22 to 28 d of age).

Treatment (Ambient temperature was 32 °C from 7 to 28 d of age).

However, there was also a significant difference in the prevalence of *Salmonella* in the crop (P = 0.0001) and the liver-spleen (P = 0.006) of the contact birds. The prevalence of the pathogen in the crops (83.3 %) and liver-spleen (44.4 %) of the contact birds reared under normal ambient temperature was higher than in the birds reared in the elevated ambient temperature. But the prevalence and the concentration of the pathogen in the ceca of the contact birds were not the same (P > 0.05). The detail of the result of the *Salmonella* analyses on contact birds is in Table 15.

Table 15. Prevalence of *Salmonella* in the organs and concentration (log_{10} CFU/ g) in the ceca of the contact birds reared under either normal or elevated ambient temperature for their age

Group	Crop/ Total crop (%)	Liver-spleen/ Total Liver-spleen (%)	Ceca/ Total Ceca (%)	Infected bird/ Total bird (%)	*Salmonella* (log_{10} CFU/ g)in the cecal content
Control	30/36 (83.3)[a]	16/ 36 (44.4)[a]	29/36 (80.6)[a]	36/ 36 (100)[a]	3.69 ± 0.25[a]
Treatment	15/ 39 (38.5)[b]	6/39 (15.4)[b]	30/ 39 (76.9)[a]	36/39 (92.3)[a]	3.40 ± 0.23[a]

Numbers with same superscript letter a-b across the column are not significantly different

Not significantly different when P > 0.05.

Control (Ambient temperature was 32 °C from 7 to 14 d of age, 29 °C from 15 to 21 d of age and 26 °C from 22 to 28 d of age).

Treatment (Ambient temperature was 32 °C from 7 to 28 d of age).

Environmental conditions have been identified as one of the factors that may increase the prevalence of *Salmonella* in the poultry. Rearing of poultry in an unsuitable environment such as one with elevated temperature may reduce resistance of birds to infection. Birds reared under high temperatures are likely to experience heat stress and are more susceptible to *Salmonella* infection (Bailey, 1988; Burkholder, et al., 2008). But in this study rearing birds under an elevated temperature condition did not result to an increase in the prevalence of *Salmonella* in the crop and the liver-spleen. Contact birds were exposed to the pathogen through the fecal shedding of *Salmonella* in the litter

by the seeder birds, observation of the birds in the temperature elevated room suggested lower foraging activities by the birds. Birds in the temperature elevated room spent most time on sitting, and stretching their legs and wings. Whereas the birds reared in the room with normal ambient temperature for their age spent most of their time feeding, drinking and exhibiting other foraging such as litter pecking. The difference in the behavior of the birds in the room may have been responsible for the difference between the prevalence of *Salmonella* in the crop and the liver-spleen of the contact birds in the study.

There was no difference in the heterophil-lymphocyte ratio content of the blood of the birds on 10 d (Table 16) when the ambient temperature in the rooms where both groups of birds were reared was the same (32 °C). However, there was a significant difference ($P = < 0.0001$) in the heterophil-lymphocyte ratio in the blood sampled on 25 d of the study. The mean (0.423) of the blood heterophil-lymphocyte ratio of the birds in the treatment group (ambient temperature 32 °C) was higher than the mean (0.227) of the birds in the control group (ambient temperature 26 °C). This finding is consistent with the reports studies that suggested an increase in the circulating stress hormones in heat stressed birds (Garriga, et al., 2006; Star, et al., 2008).

Table 16. The heterophil/lymphocyte ratio content of blood of birds reared under either normal or elevated ambient temperature at 10 and 25 d of age

Group	Heterophil-lymphocyte ratio of seeder birds on 10 d	Heterophil-lymphocyte ratio on 25 d
Control	0.170 ± 0.020^{a}	0.227 ± 0.024^{b}
Treatment	0.170 ± 0.020^{a}	0.423 ± 0.036^{a}

Numbers same superscript letter a-b across the column are not significantly different.

Significant difference was when P value < 0.05.

Control (Ambient temperature was 32 °C from 7 to 14 d of age, 29 °C from 15 to 21 d of age and 26 °C from 22 to 28 d of age).

Treatment (Ambient temperature was 32 °C from 7 to 28 d of age).

The ratio (heterophil-lymphocyte) has been used over the past years as an indicator of physiological stress in birds (Gross and Siegel, 1983). There will be an increase in the stress hormone in the circulating plasma of an animal subjected to stressful condition (Cannon, et al., 1929; Selye, 1936). Stress hormones such as corticosterone depresses the activities of lymphoid tissues and reduce the concentration of the lymphocyte cell content of blood (Gross, et al., 1980; Scanes, 2016). Stressed birds are also immunocompromised (Smith, 2003; Thaxton and Siegel, 1970). However, findings of studies have suggested that exposure of birds to heat stress may not necessarily lower adversely reduce their immune function. The immune capability of heat stress birds were demonstrated in studies where the titer values of heat stressed birds were shown to be same as those on unstressed birds (Donker, et al., 1990; Regnier, et al., 1980). While good poultry management is very important for the general wellbeing of poultry, the result of this study suggested that heat stressed bird may not have higher susceptible to

Salmonella infection. This study is also consistent with study a report that indicated that optimally stressed birds had an increase resistance to infectious agents such as *E. coli* and Newcastle disease (Gross, et al., 1980).

There was also no significant difference between the relative weight of the cecal tonsil and the adrenal glands of the birds reared in either of the ambient room. Heat stress may cause reduction in the relative weight of lymphoid tissues in birds (Quinteiro-Filho, et al., 2010; Smith, 2003), these findings were not consistent with the result of this which suggested that ambient temperature condition may not affect the relative weight of cecal tonsil. Effect of stress on birds may depend on the severity of the stress (Siegel, 1995). The difference in the result of this study when compared to the Quinteior-Filho, et al. (2010) and Smith (2003) on effect of heat stress on the lymphoid tissues of birds might be due to the difference in the heat temperature and the experimental design.

An increase in the weight of the adrenal glands has been associated with stressed birds (Freeman, et al., 1981; Siegel, 1959), this study suggested that heat stress may not affect the relative weight of the adrenal glands. Meanwhile, this study is consist with other report which suggested that heat stress may not have effect on the size of the adrenal glands of birds subjected to elevated temperature of 37 °C for 7 d (Beuving and Vonder, 1978). Some factors may affect the response of animals to heat stress. Age (Beuving and Vonder, 1978; Blecha, et al., 1983), traits (Soleimani, et al., 2011), human-animal interaction (Hemsworth, et al., 1981), social relationships (Henry, 1992) and experience (Mason, et al., 1991; Moberg, 1985; Olanrewaju, et al., 2008) are modifiers of stress response in animals. Differences in these parameters among the birds

used in this study and in the other studies reported may have played roles in the agreement/ disparities in the results.

The result of this study also revealed that different ambient temperature in the poultry houses may have an effect on production performance of the birds (Table 17). In this study, there was a significant difference (P = 0.0002) between the live weight of the birds reared under different ambient temperature from 2 to 4 wk of age. The mean (1.172 kg) of the weight of the birds reared under normal ambient temperature prior to euthanasia was significantly higher than the mean (1.049 kg) of the weight of the birds in the elevated room temperature. This result of this study is consistent with the results other studies that showed that growth was depressed in heat stressed birds (Bray, 1983; Donkoh, 1989; Mashaly, et al., 2004; Quinteiro-Filho, et al., 2012b).

Conclusion

Rearing of broiler chicks at elevated temperature did not increase the incidence of *Salmonella* in the crops and liver-spleen of infection birds in comparison to the birds reared under normal ambient temperature. Birds reared under elevated ambient temperature were heat stressed and the growth of these heat stressed birds was depressed. While rearing birds in an elevated ambient temperature did not pose higher food safety risk, the wellbeing and the productivity performance of these birds may be negatively impacted.

Table 17. The cecal tonsil/live weight, adrenal gland/live weight ratio and live weight (Kg) of birds reared under either normal or elevated ambient temperature at 28 d of age

Group	Cecal tonsil/ live weight ratio	Adrenal gland/ live weight ratio	Live weight (Kg)
Control	$5.056 \times 10^{-4} \pm 1.704 \times 10^{-5a}$	$7.236 \times 10^{-5} \pm 8.925 \times 10^{-6a}$	1.172 ± 0.025^{a}
Treatment	$4.653 \times 10^{-4} \pm 1.913 \times 10^{-5a}$	$5.718 \times 10^{-5} \pm 6.314 \times 10^{-6a}$	1.049 ± 0.019^{b}

Numbers with same superscript letter a-b across the column are not significantly different

Significant difference was when P value < 0.05.

Control (Ambient temperature was 32 °C from 7 to 14 d of age, 29 °C from 15 to 21 d of age and 26 °C from 22 to 28 d of age).

Treatment (Ambient temperature was 32 °C from 7 to 28 d of age).

CHAPTTER VII

SUMMARY

A probiotic product identified as *Lactobacillus animalis* KTC 3501 was used as a feed additive in the feed fed to 30 hens (treatment group) from 16 – 42 wk of age in ratio 2.6:1 g/kg probiotic product to standard laying hen feed. All the hens (30 each in the treatment group and control group) used in the study were orally challenged with 9.99 $\log_{10}$ of antibiotics resistant *Salmonella* Enteritidis at 18 wk of age and at every 6 wk interval for 6 month. On weekly basis, eggs, cecal dropping drops were sampled from both the probiotic fed and the control feed fed hens were analyzed for *Salmonella*, biweekly feed samples and the original probiotic product were analyzed for *Lactobacilli*. Meanwhile, two months after the start of the study 5 birds from each of the groups were euthanized and the *Lactobacilli* spp. in their cecal, feeds and the probiotic product were identified using both Sanger and pyrosequencing. At the end of the study, all the remaining 25 hens per group were euthanized, the organs (ceca, liver-spleen and ovary) were assayed for *Salmonella*. The result of study indicated that there was no significant difference ($P > 0.05$) between the prevalence of *Salmonella* in the eggs, organs, and the cecal droppings of birds from both groups. The concentration of the pathogen in the cecal droppings and contents of the hens from both group was not significantly different as well. Also none of the *Lactobacilli* detected in the feeds and the ceca of the birds were identical to *Lactobacillus animalis* KTC 3501. The conclusion of this study was that at

the concentration of the probiotic product in the feed, the laying hens were not protected from *Salmonella* infection, the probiotic product did not prevent *Salmonella* contamination of the eggs layed by the hens fed this probiotic product. And the *Lactobacillus animalis* KTC 3501fed to the laying hens was not detected in the ceca of the hens.

The effect of rearing young broiler birds with light intensity of 5 and 50 lux from 3 to 20 d of age on horizontal transmission of *Salmonella* was tested. Two trials of the study were conducted, during each trial, 100 Salmonella free day old broiler chicken were divided into 4 pens (25 birds/ pen) with 2 pens per room. At 3 d of age 5 birds (seeder birds) from a pen per room was orally challenged with 7.7 $\log_{10}$ of antibiotics resistant *Salmonella* Typhimurium, all the birds were returned to their respective pens, and the light intensity of the rooms were reduced to either 5 or 50 lux. At 10 and 20 d of age, blood samples of the 5 *Salmonella* challenged birds, and 5 birds each from 2 other pens without *Salmonella* challenged birds were analyzed for leukocyte cell and heterophil/lymphocyte ratio concentration. Also between 10 to 20 d age the motor activities of the birds in the pens containing the Salmonella challenged birds were measured using passive infrared detector. All the birds in the 2 pens housing the *Salmonella* challenged birds and 5 birds each from the 2 pens housing the unchallenged birds were euthanized, necropsied and the organs (crop, liver-spleen and ceca) were assayed for *Salmonella*. There was no significant difference between the prevalence and concentration of *Salmonella* in the organs of the seeder birds reared under any of the light intensity, there was also no difference between the prevalence of *Salmonella* in all

the organs of the contact birds irrespective of the light intensity in the pens ($P > 0.05$). But the mean of the concentration of *Salmonella* in the cecal contents of the contact birds reared under 50 lux was significantly higher than in the birds reared under 5 lux ($P = 0.019$). The motion activities, leukocyte and heterophil/lymphocyte ratio blood concentration of birds either 5 or 50 lux were not different ($P > 0.05$). It could be concluded that rearing birds under light intensity of 5 lux may have reduced the concentration of *Salmonella* in birds. Rearing birds in pens with 5 lux did not adversely affect the health and welfare of the birds.

The effect of the lighting schedule on *Salmonella* transmission among birds reared in the same pen up to 20 d was studied. The study was conducted in duplicate on day old birds, 25 birds pen, the lighting schedules effect studied on *Salmonella* transmission among birds was continuous lighting (16L:8D) and intermittent lighting (4L:2D:4L:2D:4L:2D:4L:2D). At 3 d of age, 5 birds (seeder birds) per pen were orally challenged with 8.02 $\log_{10}$ CFU/ mL of antibiotics resistant *Salmonella* Typhimurium, returned to their respective pen, the lighting schedule in all the pens was 23L:1D with light intensity of 145 175 lux. From 10 to 20 d of the study, the lighting schedule was changed to either continuous or intermittent lighting. Blood samples of the *Salmonella* challenged birds were also assayed for leukocyte and heterophil/lymphocyte ratio concentration on both 10 and 20 of the study. At the end of the study (20 d) all the birds were euthanized, necropsied and the organs (crop, liver-spleen and ceca) were tested for *Salmonella*. The result of the study revealed that the prevalence and the mean concentration of *Salmonella* of the seeder birds in the rooms lit using either of the two

light schedule were same ($P > 0.05$), also the prevalence of pathogen in the crop and the liver-spleen of the contact birds in the rooms was not affected by light schedule. However, the prevalence and concentration of the pathogen in the ceca of the contact birds were affected by light schedule, with higher ($P < 0.05$) prevalence and concentration ($P = 0.0002$ and < 0.0001 respectively) of the pathogen observed in the ceca of the contact birds reared in the room lit using continuous lighting schedule. The indicators of stress (leukocyte and heterophil/lymphocyte ratio concentration of blood) were not affected by lighting schedule. This study further confirmed that poultry production practices may affect the poultry food safety, intermittent lighting schedule may the presence of *Salmonella* in the ceca of birds.

Heat management effect on *Salmonella* transmission was examined in birds from 2 to 4 wk of age. Two heat management techniques were used in the study, Total of one hundred Ross 708 broiler chicks of day old were divided into 4 pens, and 2 pens per heat treatment. Five birds (seeder birds) per pen were orally challenged with 8.33 $\log_{10}$ CFU/ mL of antibiotics resistant *Salmonella* Typhimurium and were returned to their respective pens. The temperature in all the pens was 35º C from 0 to 7 d of age, this was reduced to 32º C at 7d of age. The temperature in the normal ambient temperature pens were further reduced by 3º C at 14 and 21 d of age, while the ambient temperature in the in the elevated temperature pens remained at 32º C throughout the reminding period of the study. Blood samples of all the birds were collected and analyzed at 10 and 25 d of age. At the end of the study (28 d), each of the birds was weighed euthanized, necropsied. Adrenal gland and ceca tonsil of each bird were weighed separately. Organs

(crop, liver-spleen and ceca) of each bird were analyzed for *Salmonella*. The prevalence and concentration of *Salmonella* between the organs of the seeder birds in either of the two ambient temperatures were not significantly different (P> 0.05), similarly there no difference in the prevalence and concentration of *Salmonella* in the ceca of the contact birds. There was significant difference between the prevalence of the pathogen in the crop (P = 0.0001) and the liver-spleen (P = 0.006) of the contact birds. There was higher prevalence of the pathogen in the crop (83.3 %) and liver-spleen (44.4 %) of the birds reared in the pens with normal ambient temperature. The study also showed that at 25 d of the study, the heterophil/lymphocyte ratio was significantly higher (P = <0.0001) in the birds reared in an elevated ambient temperature and the weight of this group of bird was significantly lower (P = 0.0002). This study demonstrated that when birds were reared in an elevated ambient temperature condition, the birds may experience physiological stress which might affect their productivity performance, but may not have effect on the *Salmonella* infection status.

REFERENCES

Abdulamir, A. S., T. Yoke, N. Nordin, and F. A. Bakar. 2010. Detection and quantification of probiotic bacteria using optimized DNA extraction, traditional and real-time PCR methods in complex microbial communities. African Journal of Biotechnology 9:1481-1492.

Abed, T. A. 2013. Evaluation of methods for the extraction and purification of DNA of cultured *Lactobacillus* colony isolated from dairy products. Int J Appl Microbiol Biotechnol Res 1:20-25.

Adler, H., and A. DaMassa. 1980. Effect of ingested lactobacilli on *Salmonella* Infantis and *Escherichia coli* and on intestinal flora, pasted vents, and chick growth. Avian Diseases:868-878.

Akoy, R. A. M. 2015. The effects of probiotics, prebiotics and synbiotics on gut flora, immune function and blood characteristics of broilers. School of Biological Science, Plymouth University, Plymouth, England.

Al-Fataftah, A.-R. A., and Z. Abu-Dieyeh. 2007. Effect of chronic heat stress on broiler performance in Jordan. International Journal of Poultry Science 6:64-70.

Al-Homidan, A. 2004. 2004 Spring meeting of the WPSA UK Branch posters: Effect of temperature and light regimen on ammonia, dust concentrations and broiler performance. British Poultry Science 45:S35-S36.

Allen, V., F. Fernandez, and M. Hinton. 1997. Evaluation of the influence of supplementing the diet with mannose or palm kernel meal on *Salmonella* colonisation in poultry. British Poultry Science 38:485-488.

Alvino, G., R. Blatchford, G. Archer, and J. Mench. 2009. Light intensity during rearing affects the behavioural synchrony and resting patterns of broiler chickens. British Poultry Science 50:275-283.

American Veterinary Medical Association. 2007. AVMA guidelines on euthanasia. American Veterinary Medical Association.

Andrews, W., P. Poelma, C. Wilson, and A. Romero. 1978. Isolation and identification of *Salmonella*. Bacteriological analytical manual, 5th ed. Association of Official Analytical Chemists, Washington, DC:1-29.

Appleby, M. C., B. O. Hughes, and H. A. Elson. 1992. Poultry production systems. Behaviour, management and welfare. CAB international.

Arnon, R., M. Shapira, and C. O. Jacob. 1983. Synthetic vaccines. Journal of Immunological Methods 61:261-273.

Atterbury, R., R. Davies, J. Carrique-Mas, V. Morris, D. Harrison, V. Tucker, and V. Allen. 2010. Effect of delivery method on the efficacy of *Salmonella* vaccination in chickens. Veterinary Record: Journal of the British Veterinary Association 167.

Axelsson, L., T. Chung, W. Dobrogosz, and S. Lindgren. 1989. Production of a broad spectrum antimicrobial substance by *Lactobacillus reuteri*. Microbial Ecology in Health and Disease 2:131-136.

Azad, M., M. Kikusato, T. Maekawa, H. Shirakawa, and M. Toyomizu. 2010. Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens exposed to chronic heat stress. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 155:401-406.

Babu, U., R. Dalloul, M. Okamura, H. Lillehoj, H. Xie, R. Raybourne, D. Gaines, and R. Heckert. 2004. *Salmonella* Enteritidis clearance and immune responses in chickens following *Salmonella* vaccination and challenge. Veterinary Immunology and Immunopathology 101:251-257.

Bailey, J., L. Blankenship, and N. Cox. 1991. Effect of fructooligosaccharide on *Salmonella* colonization of the chicken intestine. Poultry Science 70:2433-2438.

Bailey, J., N. Stern, P. Fedorka-Cray, S. Craven, N. Cox, D. Cosby, S. Ladely, and M. Musgrove. 2001. Sources and movement of *Salmonella* through integrated poultry operations: a multistate epidemiological investigation. Journal of Food Protection® 64:1690-1697.

Bailey, J. S. 1988. Integrated colonization control of *Salmonella* in poultry. Poultry Science 67:928-932.

Bailey, J. S. 1993. Control of *Salmonella* and *Campylobacter* in poultry production. A summary of work at Russell Research Center. Poultry Science 72:1169-1173.

Baird-Parker, A. 1990. Foodborne salmonellosis. The Lancet 336:1231-1235.

Barbour, E., W. Frerichs, N. Nabbut, P. Poss, and M. Brinton. 1993. Evaluation of bacterins containing three predominant phage types of *Salmonella* Enteritidis for

prevention of infection in egg-laying chickens. American Journal of Veterinary Research 54:1306-1309.

Barnes, E. M., G. Mead, D. Barnuml, and E. Harry. 1972. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. British Poultry science 13:311-326.

Barnhart, E., D. Caldwell, M. Crouch, J. Byrd, D. Corrier, and B. Hargis. 1999. Effect of lactose administration in drinking water prior to and during feed withdrawal on *Salmonella* recovery from broiler crops and ceca. Poultry Science 78:211-214.

Barrow, P., J. Hassan, and A. Berchieri. 1990. Reduction in faecal excretion of *Salmonella* Typhimurium strain F98 in chickens vaccinated with live and killed *S*. Typhimurium organisms. Epidemiology and Infection 104:413-426.

Barrow, P., and M. A. Lovell. 1991. Experimental infection of egg-laying hens with *Salmonella* Enteritidis phage type 4. Avian Pathology 20:335-348.

Belay, T., and G. Sonnenfeld. 2002. Differential effects of catecholamines on in vitro growth of pathogenic bacteria. Life Sciences 71:447-456.

Beuving, G., and G. Vonder. 1978. Effect of stressing factors on corticosterone levels in the plasma of laying hens. General and Comparative Endocrinology 35:153-159.

Bhatia, T., G. McNabb, H. Wyman, and G. Nayar. 1979. *Salmonella* isolation from litter as an indicator of flock infection and carcass contamination. Avian diseases:838-847.

Bichler, L., K. Nagaraja, and D. Halvorson. 1996. *Salmonella* Enteritidis in eggs, cloacal swab specimens, and internal organs of experimentally infected white leghorn chickens. American Journal of Veterinary Research 57:489-495.

Blatchford, R., G. Archer, and J. Mench. 2012. Contrast in light intensity, rather than day length, influences the behavior and health of broiler chickens. Poultry Science 91:1768-1774.

Blatchford, R., K. Klasing, H. Shivaprasad, P. Wakenell, G. Archer, and J. Mench. 2009. The effect of light intensity on the behavior, eye and leg health, and immune function of broiler chickens. Poultry Science 88:20-28.

Blecha, F., D. Pollman, and D. Nichols. 1983. Weaning pigs at an early age decreases cellular immunity. Journal of Animal Science 56:396-400.

Bolder, N., J. Wagenaar, F. Putirulan, K. Veldman, and M. Sommer. 1999. The effect of flavophospholipol (Flavomycin) and salinomycin sodium (Sacox) on the excretion of *Clostridium perfringens, Salmonella* Enteritidis, and *Campylobacter jejuni* in broilers after experimental infection. Poultry Science 78:1681-1689.

Bonnet, S., P. Geraert, M. Lessire, B. Carre, and S. Guillaumin. 1997. Effect of high ambient temperature on feed digestibility in broilers. Poultry Science 76:857-863.

Boshouwers, F., and E. Nicaise. 1993. Artificial light sources and their influence on physical activity and energy expenditure of laying hens. British Poultry Science 34:11-19.

Braden, C. R. 2006. *Salmonella* enterica serotype Enteritidis and eggs: a national epidemic in the United States. Clinical Infectious Diseases 43:512-517.

Brambell, F. W. R. 1970. Report of the technical committee to enquire into the welfare of animals kept under intensive livestock husbandry systems: presented to parliament by the secretary of state for Scotland and the Minister of Agriculture, Fisheries and Food by Command of her Majesty December, 1965. HM Stationery Office.

Bray, T. 1983. Broilers—Why temperature is important. Gleadthorpe Experimental Husbandry Farm Poultry Booklet:10-21.

Bruce, R. A., L. A. Achenbach, and J. D. Coates. 1999. Reduction of (per) chlorate by a novel organism isolated from paper mill waste. Environmental Microbiology 1:319-329.

Bryan, E. H., and G. A. Rohlich. 1954. Biological reduction of sodium chlorate as applied to measurement of sewage BOD. Sewage and Industrial Wastes:1315-1324.

Bryan, F. L. 1980. Foodborne diseases in the United States associated with meat and poultry. Journal of Food Protection® 43:140-150.

Bryan, F. L., and M. P. Doyle. 1995. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. Journal of Food Protection® 58:326-344.

Burkholder, K., K. Thompson, M. Einstein, T. Applegate, and J. Patterson. 2008. Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to *Salmonella* Enteritidis colonization in broilers. Poultry Science 87:1734-1741.

Buyse, J., P. Simons, F. Boshouwers, and E. Decuypere. 1996. Effect of intermittent lighting, light intensity and source on the performance and welfare of broilers. World's Poultry Science Journal 52:121-130.

Bygrave, A., and J. Gallagher. 1989. Transmission of *Salmonella* Enteritidis in poultry. Veterinary Record 124:571-571.

Byrd, J., R. Anderson, T. Callaway, R. Moore, K. Knape, L. Kubena, R. Ziprin, and D. Nisbet. 2003. Effect of experimental chlorate product administration in the drinking water on *Salmonella* Typhimurium contamination of broilers. Poultry Science 82:1403-1406.

Byrd, J., M. Burnham, J. McReynolds, R. Anderson, K. Genovese, T. Callaway, L. Kubena, and D. Nisbet. 2008. Evaluation of an experimental chlorate product as a preslaughter feed supplement to reduce *Salmonella* in meat-producing birds. Poultry Science 87:1883-1888.

Byrd, J., J. DeLoach, D. Corrier, D. Nisbet, and L. Stanker. 1999. Evaluation of *Salmonella* serotype distributions from commercial broiler hatcheries and grower houses. Avian Diseases:39-47.

Byrd, J., B. Hargis, D. Caldwell, R. Bailey, K. Herron, J. McReynolds, R. Brewer, R. Anderson, K. Bischoff, and T. Callaway. 2001. Effect of lactic acid administration in the drinking water during preslaughter feed withdrawal on *Salmonella* and *Campylobacter* contamination of broilers. Poultry Science 80:278-283.

Bywater, R. 2004. Veterinary use of antimicrobials and emergence of resistance in zoonotic and sentinel bacteria in the EU. Journal of Veterinary Medicine, Series B 51:361-363.

Calvet, S., H. Van den Weghe, R. Kosch, and F. Estellés. 2009. The influence of the lighting program on broiler activity and dust production. Poultry Science 88:2504-2511.

Campo, J., M. Gil, S. Dávila, and I. Muñoz. 2007. Effect of lighting stress on fluctuating asymmetry, heterophil-to-lymphocyte ratio, and tonic immobility duration in eleven breeds of chickens. Poultry Science 86:37-45.

Cannon, W. B., H. Newton, E. Bright, V. Menkin, and R. Moore. 1929. Some aspects of the physiology of animals surviving complete exclusion of sympathetic nerve impulses. American Journal of Physiology--Legacy Content 89:84-107.

Carrique-Mas, J., M. Breslin, L. Snow, I. McLaren, A. Sayers, and R. Davies. 2009. Persistence and clearance of different *Salmonella* serovars in buildings housing laying hens. Epidemiology and Infection 137:837-846.

Carter, A. J., M. R. Adams, M. J. Woodward, and R. M. La Ragione. 2009. Control strategies for *Salmonella* colonization of poultry: The probiotic perspective. Food Science and Technology 5:103-115.

Cason, J., N. Cox, and J. Bailey. 1994. Transmission of *Salmonella* Typhimurium during hatching of broiler chicks. Avian Diseases:583-588.

Castanon, J. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poultry Science 86:2466-2471.

Centers for Disease Control and Prevention 2015. Reports of selected *Salmonella* outbreak investigations from 2006 to 2015. http://www.cdc.gov/*salmonella*/outbreaks.html.

Chancellor, L., and B. Glick. 1960. Effect of temperature as a stresser on white blood cells, adrenals and bursae of Fabricius of chicks. American Journal of Physiology--Legacy Content 198:1346-1348.

Cherrington, C. A., M. Hinton, G. Pearson, and I. Chopra. 1991. Short-chain organic acids at pH 5.0 kill *Escherichia coli* and *Salmonella* spp. without causing membrane perturbation. Journal of Applied Bacteriology 70:161-165.

Chittick, P., A. Sulka, R. V. Tauxe, and A. M. Fry. 2006. A summary of national reports of foodborne outbreaks of *Salmonella* Heidelberg infections in the United States: clues for disease prevention. Journal of Food Protection® 69:1150-1153.

Classen, H., C. Riddell, and F. Robinson. 1991. Effects of increasing photoperiod length on performance and health of broiler chickens. British Poultry Science 32:21-29.

Coates, M., M. Davies, and S. Kon. 1955. The effect of antibiotics on the intestine of the chick. British Journal of Nutrition 9:110-119.

Cogan, T., and T. Humphrey. 2003. The rise and fall of *Salmonella* Enteritidis in the UK. Journal of Applied Microbiology 94:114-119.

Collins, F. 1972. Effect of adjuvant on immunogenicity of a heat-killed *Salmonella* vaccine. Journal of Infectious Diseases 126:69-76.

Collins, M. D., and G. R. Gibson. 1999. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. The American Journal of Clinical Nutrition 69:1052s-1057s.

Corrier, D., J. Byrd, B. Hargis, M. Hume, R. Bailey, and L. Stanker. 1999. Survival of *Salmonella* in the crop contents of market-age broilers during feed withdrawal. Avian Diseases:453-460.

Corrier, D. E., A. Hinton Jr, R. L. Ziprin, R. C. Beier, and J. R. DeLoach. 1990. Effect of dietary lactose on cecal pH, bacteriostatic volatile fatty acids, and *Salmonella* Typhimurium colonization of broiler chicks. Avian Diseases:617-625.

Cox, N., J. Bailey, J. Mauldin, and L. Blankenship. 1990. Research note: Presence and impact of *Salmonella* contamination in commercial broiler hatcheries. Poultry Science 69:1606-1609.

Curtiss, R., S. M. Kelly, and J. O. Hassan. 1993. Live oral avirulent *Salmonella* vaccines. Veterinary Microbiology 37:397-405.

Dale, N., and H. Fuller. 1980. Effect of diet composition on feed intake and growth of chicks under heat stress. II. Constant vs. cycling temperatures. Poultry Science 59:1434-1441.

Davies, R., and M. Breslin. 2003a. Observations on the distribution and persistence of *Salmonella enterica* serovar Enteritidis phage type 29 on a cage layer farm before and after the use of competitive exclusion treatment. British Poultry Science 44:551-557.

Davies, R., and M. Breslin. 2004. Observations on *Salmonella* contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella enterica* serovar Enteritidis had been used. Avian Pathology 33:133-144.

Davies, R., M. Breslin, J. Corry, W. Hudson, and V. Allen. 2001. Observations on the distribution and control of *Salmonella* species in two integrated. Veterinary Record 149:227-232.

Davies, R. H., and M. Breslin. 2003b. Persistence of *Salmonella* Enteritidis Phage Type 4 in the environment and arthropod vectors on an empty free-range chicken farm. Environmental Microbiology 5:79-84.

Davison, S., C. E. Benson, D. J. Henzler, and R. J. Eckroade. 1999. Field observations with *Salmonella* enteritidis bacterins. Avian Diseases:664-669.

De Buck, J., F. Van Immerseel, F. Haesebrouck, and R. Ducatelle. 2004. Colonization of the chicken reproductive tract and egg contamination by *Salmonella*. Journal of Applied Microbiology 97:233-245.

De Groot, G., and A. Stouthamer. 1969. Regulation of reductase formation in *Proteus mirabilis*. Archiv für Mikrobiologie 66:220-233.

De Reu, K., K. Grijspeerdt, W. Messens, M. Heyndrickx, M. Uyttendaele, J. Debevere, and L. Herman. 2006. Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including *Salmonella* Enteritidis. International Journal of Food Microbiology 112:253-260.

Deaton, J., F. Reece, J. McNaughton, and B. Lott. 1981. Effect of light intensity and low-level intermittent lighting on broiler performance during a high density limited-area brooding period. Poultry Science 60:2385-2387.

Deep, A., C. Raginski, K. Schwean-Lardner, B. Fancher, and H. Classen. 2013. Minimum light intensity threshold to prevent negative effects on broiler production and welfare. British Poultry Science 54:686-694.

Deep, A., K. Schwean-Lardner, T. Crowe, B. Fancher, and H. Classen. 2010. Effect of light intensity on broiler production, processing characteristics, and welfare. Poultry Science 89:2326-2333.

Deep, A., K. Schwean-Lardner, T. G. Crowe, B. I. Fancher, and H. L. Classen. 2012. Effect of light intensity on broiler behaviour and diurnal rhythms. Applied Animal Behaviour Science 136:50-56.

Denbow, D., A. Leighton, and R. Hulet. 1990. Effect of light sources and light intensity on growth performance and behaviour of female turkeys. British Poultry Science 31:439-443.

Dent, V., and R. Williams. 1982. *Lactobacillus animalis* sp. nov., a new species of *Lactobacillus* from the alimentary canal of animals. Zentralblatt für Bakteriologie Mikrobiologie und Hygiene: I. Abt. Originale C: Allgemeine, angewandte und ökologische Mikrobiologie 3:377-386.

Dewaele, I., H. Van Meirhaeghe, G. Rasschaert, M. Vanrobaeys, E. De Graef, L. Herman, R. Ducatelle, M. Heyndrickx, and K. De Reu. 2012. Persistent *Salmonella* Enteritidis environmental contamination on layer farms in the context of an implemented national control program with obligatory vaccination. Poultry Science 91:282-291.

Dhabhar, F. S. 2002. Stress-induced augmentation of immune function—the role of stress hormones, leukocyte trafficking, and cytokines. Brain, Behavior, and Immunity 16:785-798.

Dhabhar, F. S., A. H. Miller, M. Stein, B. S. McEwen, and R. L. Spencer. 1994. Diurnal and acute stress-induced changes in distribution of peripheral blood leukocyte subpopulations. Brain, Behavior, and Immunity 8:66-79.

Dietert, R. R., K. A. Golemboski, and R. E. Austic. 1994. Environment-immune interactions. Poultry Science 73:1062-1076.

Donker, R., M. Neeuwland, and A. Van Der Zijpp. 1990. Heat-stress influences on antibody production in chicken lines selected for high and low immune responsiveness. Poultry Science 69:599-607.

Donkoh, A. 1989. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. International Journal of Biometeorology 33:259-265.

Durant, J. A., D. E. Corrier, J. A. Byrd, L. H. Stanker, and S. C. Ricke. 1999. Feed deprivation affects crop environment and modulates *Salmonella* Enteritidis colonization and invasion of Leghorn hens. Applied and Environmental Microbiology 65:1919-1923.

Eeckhaut, V., F. Van Immerseel, J. Dewulf, F. Pasmans, F. Haesebrouck, R. Ducatelle, C. Courtin, J. Delcour, and W. Broekaert. 2008. Arabinoxylooligosaccharides from wheat bran inhibit *Salmonella* colonization in broiler chickens. Poultry Science 87:2329-2334.

Elkheir, S., M. Mohammed, M. Ahmed, and S. A. Gadir. 2008. Effect of feed restriction and ascorbic acid supplementation on performance of broiler chicks reared under heat stress. Research Journal of Animal and Veterinary Sciences 3:1-8.

Ellen, H., R. Bottcher, E. Von Wachenfelt, and H. Takai. 2000. Dust levels and control methods in poultry houses. Journal of Agricultural Safety and Health 6:275.

Evangelisti, D., A. English, A. Girard, J. Lynch, and I. Solomons. 1975. Influence of subtherapeutic levels of oxytetracycline on *Salmonella* Typhimurium in swine, calves, and chickens. Antimicrobial Agents and Chemotherapy 8:664-672.

Fanelli, M., W. Sadler, and J. Brownell. 1970. Preliminary studies on persistence of *Salmonellae* in poultry litter. Avian Diseases:131-141.

Fernández-Rubio, C., C. Ordóñez, J. Abad-González, A. Garcia-Gallego, M. P. Honrubia, J. J. Mallo, and R. Balaña-Fouce. 2009. Butyric acid-based feed additives help protect broiler chickens from *Salmonella* Enteritidis infection. Poultry Science 88:943-948.

Ferro, P. J., C. L. Swaggerty, P. Kaiser, I. Y. Pevzner, and M. H. Kogut. 2004. Heterophils isolated from chickens resistant to extra-intestinal *Salmonella* Enteritidis infection express higher levels of pro-inflammatory cytokine mRNA following infection than heterophils from susceptible chickens. Epidemiology and Infection 132:1029-1037.

Foley, S. L., R. Nayak, I. B. Hanning, T. J. Johnson, J. Han, and S. C. Ricke. 2011. Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. Applied and Environmental Microbiology 77:4273-4279.

Food Safety and Inspection Services. 2009. PR/HACCP Salmonella Verification, 2009. In: A comparison of *Salmonella* serotype incidence in FSIS-regulated products and salmonellosis cases.

FoodNet 2010. Preliminary FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food --- 10 States, 2009. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5914a2.htm.

Ford, A. M., D. J. Fagerberg, C. L. Quarles, B. A. George, and G. A. McKinley. 1981. Influence of salinomycin on incidence, shedding, and antimicrobial resistance of *Salmonella* Typhimurium in experimentally infected broiler chicks. Poultry Science 60:2441-2453.

Freeman, B., A. Manning, and I. Flack. 1981. Photoperiod and its effect on the responses of the immature fowl to stressors. Comparative Biochemistry and Physiology Part A: Physiology 68:411-416.

Freitas Neto, O. C. d., A. L. Mesquita, J. B. d. Paiva, F. Zotesso, and A. Berchieri Júnior. 2008. Control of *Salmonella* enterica serovar Enteritidis in laying hens by inactivated *Salmonella* Enteritidis vaccines. Brazilian Journal of Microbiology 39:390-396.

Fuchs, P. C., A. L. Barry, and S. D. Brown. 1999. In vitro activities of SCH27899 alone and in combination with 17 other antimicrobial agents. Antimicrobial Agents and Chemotherapy 43:2996-2997.

Fukata, T., K. Sasai, T. Miyamoto, and E. Baba. 1999. Inhibitory effects of competitive exclusion and fructooligosaccharide, singly and in combination, on *Salmonella* colonization of chicks. Journal of Food Protection® 62:229-233.

Fuller, A., R. 1989a. Probiotics in man and animals. Journal of Applied Bacteriology 66:365-378.

Fuller, R. 1989b. Probiotics in man and animals. Journal of Applied Bacteriology 66:365-378.

Gantois, I., R. Ducatelle, F. Pasmans, F. Haesebrouck, R. Gast, T. J. Humphrey, and F. Van Immerseel. 2009. Mechanisms of egg contamination by *Salmonella* Enteritidis. FEMS Microbiology Reviews 33:718-738.

Gantois, I., R. Ducatelle, F. Pasmans, F. Haesebrouck, I. Hautefort, A. Thompson, J. Hinton, and F. Van Immerseel. 2006. Butyrate specifically down-regulates *Salmonella* pathogenicity island 1 gene expression. Applied and Environmental Microbiology 72:946-949.

García, C., J. Soriano, V. Benítez, and P. Catalá-Gregori. 2011. Assessment of *Salmonella* spp. in feces, cloacal swabs, and eggs (eggshell and content separately) from a laying hen farm. Poultry science 90:1581-1585.

Garriga, C., R. R. Hunter, C. Amat, J. M. Planas, M. A. Mitchell, and M. Moretó. 2006. Heat stress increases apical glucose transport in the chicken jejunum. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 290:R195-R201.

Garriga, M., M. Pascual, J. Monfort, and M. Hugas. 1998. Selection of *Lactobacilli* for chicken probiotic adjuncts. Journal of Applied Microbiology 84:125-132.

Gast, R. K. 2007. Serotype-specific and serotype-independent strategies for preharvest control of food-borne *Salmonella* in poultry. Avian Diseases 51:817-828.

Gast, R. K., and C. Beard. 1990. Isolation of *Salmonella* Enteritidis from internal organs of experimentally infected hens. Avian Diseases:991-993.

Gast, R. K., J. Guard-Bouldin, and P. S. Holt. 2004. Colonization of reproductive organs and internal contamination of eggs after experimental infection of laying hens with *Salmonella* Heidelberg and *Salmonella* Enteritidis. Avian Diseases 48:863-869.

Gast, R. K., R. Guraya, D. R. Jones, and K. E. Anderson. 2014. Horizontal transmission of *Salmonella* Enteritidis in experimentally infected laying hens housed in conventional or enriched cages. Poultry Science:PS4237.

Gast, R. K., B. W. Mitchell, and P. S. Holt. 1998. Airborne transmission of *Salmonella* Enteritidis infection between groups of chicks in controlled-environment isolation cabinets. Avian Diseases:315-320.

Gast, R. K., H. D. Stone, P. S. Holt, and C. Beard. 1992. Evaluation of the efficacy of an oil-emulsion bacterin for protecting chickens against *Salmonella* Enteritidis. Avian Diseases:992-999.

Genovese, K., V. Lowry, L. Stanker, and M. Kogut. 1998. Administration of *Salmonella* Enteritidis-immune lymphokine to day-old turkeys by subcutaneous, oral, and nasal routes: A comparison of effects on *Salmonella* Enteritidis liver invasion, peripheral blood heterophilia and heterophil activation. Avian Pathology 27:597-604.

Geraert, P., J. Padilha, and S. Guillaumin. 1996. Metabolic and endocrine changes induced by chronic heatexposure in broiler chickens: biological and endocrinological variables. British Journal of Nutrition 75:205-216.

Girard, A., A. English, D. Evangelisti, J. Lynch, and I. Solomons. 1976. Influence of subtherapeutic levels of a combination of neomycin and oxytetracycline on *Salmonella* Typhimurium in swine, calves, and chickens. Antimicrobial Agents and Chemotherapy 10:89-95.

Glenn, G., and M. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition 125:1401-1412.

Goodnough, M. C., and E. A. Johnson. 1991. Control of *Salmonella* Enteritidis infections in poultry by polymyxin B and trimethoprim. Applied and Environmental Microbiology 57:785-788.

Griffin, J. F. T. 1989. Stress and immunity: a unifying concept. Veterinary Immunology and Immunopathology 20:263-312.

Grimont, P. A., F. Grimont, and P. Bouvet. 2000. Taxonomy of the genus *Salmonella*. *Salmonella* in domestic animals:1-17.

Grizard, D., and C. Barthomeuf. 1999. Non-digestible oligosaccharides used as prebiotic agents: mode of production and beneficial effects on animal and human health. Reproduction Nutrition Development 39:563-588.

Gross, W., and H. Siegel. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Diseases:972-979.

Gross, W., and P. Siegel. 1985. Selective breeding of chickens for corticosterone response to social stress. Poultry Science 64:2230-2233.

Gross, W., P. Siegel, and R. DuBose. 1980. Some effects of feeding corticosterone to chickens. Poultry Science 59:516-522.

Guan, J., C. Grenier, and B. Brooks. 2006. In vitro study of *Salmonella* Enteritidis and *Salmonella* Typhimurium definitive type 104: survival in egg albumen and penetration through the vitelline membrane. Poultry Science 85:1678-1681.

Guard-Petter, J. 2001. The chicken, the egg and *Salmonella* Enteritidis. Environmental Microbiology 3:421-430.

Gusils, C., A. P. Chaia, S. Gonzalez, and G. Oliver. 1999a. *Lactobacilli* isolated from chicken intestines: potential use as probiotics. Journal of Food Protection® 62:252-256.

Gusils, C., S. N. González, and G. Oliver. 1999b. Some probiotic properties of chicken *Lactobacilli*. Canadian Journal of Microbiology 45:981-987.

Hammond, J. 1952. Physiological limits to intensive production in animals. British Agricultural Bulletin 4:222-224.

Harbaugh, E., D. Trampel, I. Wesley, S. Hoff, R. Griffith, and H. S. Hurd. 2006. Rapid aerosol transmission of *Salmonella* among turkeys in a simulated holding-shed environment. Poultry Science 85:1693-1699.

Hassan, J. O., and R. Curtiss III. 1997. Efficacy of a Live Avirulent *Salmonella* Typhimuriun Vaccine in Preventing Colonization and Invasion of Laying Hens by *Salmonella* Typhimurium and *Salmonella* Enteritidis. Avian Diseases:783-791.

Havenaar, R., and J. H. Huis. 1992. Probiotics: a general view. Pages 151-170 in The Lactic Acid Bacteria Volume 1Springer.

Hemsworth, P., J. Barnett, and C. Hansen. 1981. The influence of handling by humans on the behavior, growth, and corticosteroids in the juvenile female pig. Hormones and Behavior 15:396-403.

Henry, J. P. 1992. Biological basis of the stress response. Integrative Physiological and Behavioral Science 27:66-83.

Heres, L., B. Engel, F. Van Knapen, M. De Jong, J. Wagenaar, and H. Urlings. 2003. Fermented liquid feed reduces susceptibility of broilers for *Salmonella* Enteritidis. Poultry Science 82:603-611.

Heuer, O. E., K. Pedersen, J. Andersen, and M. Madsen. 2002. Vancomycin-resistant *Enterococci* (VRE) in broiler flocks 5 years after the avoparcin ban. Microbial Drug Resistance 8:133-138.

Higgins, J., S. Higgins, J. Vicente, A. Wolfenden, G. Tellez, and B. Hargis. 2007. Temporal effects of lactic acid bacteria probiotic culture on *Salmonella* in neonatal broilers. Poultry Science 86:1662-1666.

Higgins, J., S. Higgins, A. Wolfenden, S. Henderson, A. Torres-Rodriguez, J. Vicente, B. Hargis, and G. Tellez. 2010. Effect of lactic acid bacteria probiotic culture treatment timing on *Salmonella* Enteritidis in neonatal broilers. Poultry Science 89:243-247.

Holmes, W. a. P., JG. 1976. The adrenal cortex of birds. In: Siegel H. S. 1995. Stress, strains and resistance 1.

Holt, P. S., C. J. Geden, R. W. Moore, and R. K. Gast. 2007. Isolation of *Salmonella enterica* serovar Enteritidis from houseflies (*Musca domestica*) found in rooms containing *Salmonella* serovar Enteritidis-challenged hens. Applied and Environmental Microbiology 73:6030-6035.

Holt, P. S., B. W. Mitchell, and R. K. Gast. 1998. Airborne horizontal transmission of *Salmonella* Enteritidis in molted laying chickens. Avian Diseases:45-52.

Hosoi, T., A. Ametani, K. Kiuchi, and S. Kaminogawa. 2000. Improved growth and viability of *Lactobacilli* in the presence of *Bacillus subtilis* (natto), catalase, or subtilisin. Canadian Journal of Microbiology 46:892-897.

Howlider, M., and S. Rose. 1987. Temperature and the growth of broilers. World's Poultry Science Journal 43:228-237.

Howlider, M., and S. Rose. 1989. Rearing temperature and the meat yield of broilers. British Poultry Science 30:61-67.

Huber, G., and G. Nesemann. 1968. Moenomycin, an inhibitor of cell wall synthesis. Biochemical and Biophysical Research Communications 30:7-13.

Hughes, B., and I. Duncan. 1972. The influence of strain and environmental factors upon feather pecking and cannibalism in fowls. British Poultry Science 13:525-547.

Hughes, S., T.-Y. Poh, N. Bumstead, and P. Kaiser. 2007. Re-evaluation of the chicken MIP family of chemokines and their receptors suggests that CCL5 is the prototypic MIP family chemokine, and that different species have developed different repertoires of both the CC chemokines and their receptors. Developmental & Comparative Immunology 31:72-86.

Hume, M. E., D. E. Corrier, S. Ambrus, A. Hinton Jr, and J. R. DeLoach. 1993. Effectiveness of dietary propionic acid in controlling *Salmonella* Typhimurium colonization in broiler chicks. Avian Diseases:1051-1056.

Humphrey, T., A. Baskerville, A. Whitehead, B. Rowe, and A. Henley. 1993. Influence of feeding patterns on the artificial infection of laying hens with *Salmonella* Enteritidis phage type 4. The Veterinary Record 132:407-409.

Huth, J. C., and G. S. Archer. 2015. Comparison of two LED light bulbs to a dimmable CFL and their effects on broiler chicken growth, stress, and fear. Poultry Science 94:2027-2036.

Ishihara, N., D.-C. Chu, S. Akachi, and L. Juneja. 2000. Preventive effect of partially hydrolyzed guar gum on infection of *Salmonella* Enteritidis in young and laying hens. Poultry Science 79:689-697.

Jay, J., M. Loessner, and D. Golden. 2005. Modern food microbiology 7 thNew York, NY: Springer Since Business Media, LLC.

Jin, L., Y. Ho, N. Abdullah, and S. Jalaludin. 1997. Probiotics in poultry: modes of action. World's Poultry Science Journal 53:351-368.

Jin, L. Z., Y. W. Ho, N. Abdullah, M. Ali, and S. Jalaludin. 1996a. Antagonistic effects of intestinal *Lactobacillus* isolates on pathogens of chicken. Letters in Applied Microbiology 23:67-71.

Jin, L. Z., Y. W. Ho, M. Ali, N. Abdullah, and S. Jalaludin. 1996b. Effect of adherent *Lactobacillus* spp. on in vitro adherence of *Salmonellae* to the intestinal epithelial cells of chicken. Journal of Applied Bacteriology 81:201-206.

Jones, F., R. Axtell, D. Rives, S. Scheideler, F. Tarver, R. Walker, and M. Wineland. 1991. A survey of *Salmonella* contamination in modern broiler production. Journal of Food Protection® 54:502-513.

Jones, F., and K. Richardson. 2004. *Salmonella* in commercially manufactured feeds. Poultry Science 83:384-391.

Kallapura, G., A. Botero, S. Layton, L. Bielke, J. Latorre, A. Menconi, X. Hernández-Velasco, D. J. Bueno, B. Hargis, and G. Téllez. 2014a. Evaluation of recovery of *Salmonella* from trachea and ceca in commercial poultry. The Journal of Applied Poultry Research 23:132-136.

Kallapura, G., M. Morgan, N. Pumford, L. Bielke, A. Wolfenden, O. Faulkner, J. Latorre, A. Menconi, X. Hernandez-Velasco, and V. Kuttappan. 2014b. Evaluation of the respiratory route as a viable portal of entry for *Salmonella* in poultry via intratracheal challenge of *Salmonella* Enteritidis and *Salmonella* Typhimurium. Poultry science 93:340-346.

Keller, L. H., C. E. Benson, K. Krotec, and R. J. Eckroade. 1995. *Salmonella* Enteritidis colonization of the reproductive tract and forming and freshly laid eggs of chickens. Infection and Immunity 63:2443-2449.

Kinde, H., D. M. Castellan, D. Kerr, J. Campbell, R. Breitmeyer, and A. Ardans. 2005. Longitudinal monitoring of two commercial layer flocks and their environments for *Salmonella enterica* serovar Enteritidis and other *salmonellae*. Avian Diseases 49:189-194.

Kirby, J. D., and D. P. Froman. 1991. Research note: evaluation of humoral and delayed hypersensitivity responses in cockerels reared under constant light or a twelve hour light: twelve hour dark photoperiod. Poultry Science 70:2375-2378.

Kleessen, B., L. Hartmann, and M. Blaut. 2001. Oligofructose and long-chain inulin: influence on the gut microbial ecology of rats associated with a human faecal flora. British Journal of Nutrition 86:291-300.

Koenen, M., J. Kramer, R. Van Der Hulst, L. Heres, S. Jeurissen, and W. Boersma. 2004. Immunomodulation by probiotic *Lactobacilli* in layer-and meat-type chickens. British Poultry Science 45:355-366.

Kopanic, R. J., B. W. Sheldon, and C. Wright. 1994. Cockroaches as vectors of *Salmonella*: laboratory and field trials. Journal of Food Protection® 57:125-131.

Kwon, H.-J., and S.-H. Cho. 2011. Pathogenicity of SG 9R, a rough vaccine strain against fowl typhoid. Vaccine 29:1311-1318.

Lapuz, R., H. Tani, K. Sasai, K. Shirota, H. Katoh, and E. Baba. 2008. The role of roof rats (Rattus rattus) in the spread of *Salmonella* Enteritidis and *S*. Infantis contamination in layer farms in eastern Japan. Epidemiology and Infection 136:1235-1243.

Lara, L. J., and M. H. Rostagno. 2013. Impact of heat stress on poultry production. Animals 3:356-369.

Larbier, M., A. Chagneau, and P. Geraert. 1993. Influence of ambient temperature on true digestibility of protein and amino acids of rapeseed and soybean meals in broilers. Poultry Science 72:289-295.

Lauber, J. K. 1987. Light-induced avain glaucoma as an animal model for human primary glaucoma. Journal of Ocular Pharmacology and Therapeutics 3:77-100.

Lauber, J. K. 1991. Three Avian Eye Enlargement Protcols as Myopia Models: Effects of Pharmacological Intervention. Journal of Ocular Pharmacology and Therapeutics 7:65-75.

Lawhon, S. D., R. Maurer, M. Suyemoto, and C. Altier. 2002. Intestinal short-chain fatty acids alter *Salmonella* Typhimurium invasion gene expression and virulence through BarA/SirA. Molecular Microbiology 46:1451-1464.

Leary, S., W. Underwood, R. Anthony, S. Cartner, D. Corey, T. Grandin, C. B. Greenacre, S. Gwaltney-Bran, M. A. McCrackin, and R. Meyer. 2013. AVMA guidelines for the euthanasia of animals: 2013 edition.

Lee, Y., C. Lim, W. Teng, A. Ouwehand, E. Tuomola, and S. Salminen. 2000. Quantitative approach in the study of adhesion of lactic acid bacteria to intestinal cells and their competition with enterobacteria. Applied and Environmental Microbiology 66:3692-3697.

Leeson, S., and J. D. Summers. 2005. Commercial Poultry Nutrition. 3rd ed. University Books, Guelph, Ontario, Canada.

Lewis, K., and D. M. McKay. 2009. Metabolic stress evokes decreases in epithelial barrier function. Annals of the New York Academy of Sciences 1165:327-337.

Lewis, P., and T. Morris. 2000. Poultry and coloured light. World's Poultry Science Journal 56:189-207.

Lewis, P., and T. Morris. 2006. Poultry lighting: the theory and practice. Northcot Hampshire, UK.

Li, T., D. Troilo, A. Glasser, and H. C. Howland. 1995. Constant light produces severe corneal flattening and hyperopia in chickens. Vision Research 35:1203-1209.

Lien, R., J. Hess, S. McKee, S. Bilgili, and J. Townsend. 2007. Effect of light intensity and photoperiod on live performance, heterophil-to-lymphocyte ratio, and processing yields of broilers. Poultry Science 86:1287-1293.

Liljebjelke, K. A., C. L. Hofacre, T. Liu, D. G. White, S. Ayers, S. Young, and J. J. Maurer. 2005. Vertical and horizontal transmission of *Salmonella* within integrated broiler production system. Foodbourne Pathogens and Disease 2:90-102.

Line, J. E., J. S. Bailey, N. A. Cox, N. J. Stern, and T. Tompkins. 1998. Effect of yeast-supplemented feed on *Salmonella* and *Campylobacter* populations in broilers. Poultry Science 77:405-410.

Logan, B. E. 1998. A review of chlorate-and perchlorate-respiring microorganisms. Bioremediation Journal 2:69-79.

Lyte, M., and S. Ernst. 1992. Catecholamine induced growth of gram negative bacteria. Life Siences 50:203-212.

Mack, L., J. Felver-Gant, R. Dennis, and H. W. Cheng. 2013. Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. Poultry Science 92:285-294.

MacKenzie, M. A., and B. Bains. 1976. Dissemination of *Salmonella* serotypes from raw feed ingredients to chicken carcases. Poultry Science 55:957-960.

Malek, M., J. Hasenstein, and S. Lamont. 2004. Analysis of chicken TLR4, CD28, MIF, MD-2, and LITAF genes in a *Salmonella* Enteritidis resource population. Poultry Science 83:544-549.

Malmqvist, Å., T. Welander, and L. Gunnarsson. 1991. Anaerobic growth of microorganisms with chlorate as an electron acceptor. Applied and Environmental Microbiology 57:2229-2232.

Manning, T. S., and G. R. Gibson. 2004. Prebiotics. Best Practice & Research Clinical Gastroenterology 18:287-298.

Marin, C., S. Balasch, S. Vega, and M. Lainez. 2011. Sources of *Salmonella* contamination during broiler production in Eastern Spain. Preventive Veterinary Medicine 98:39-45.

Martin, G. 1989. Federpickhaufigkeit in Abhangigkeit von draht und Einstreuboden sowie von der Lichtintensitat: In Manser, CE. 1996. Effects of lighting on the welfare of domestic poultry: a review. Animal Welfare 5:341-360.

Mashaly, M., G. Hendricks, M. Kalama, A. Gehad, A. Abbas, and P. Patterson. 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poultry Science 83:889-894.

Mason, J. W. 1974. Specificity in the organization of neuroendocrine response profiles. Frontiers in Neurology and Neuroscience Research:68-80.

Mason, W., S. Mendoza, and G. Moberg. 1991. Persistent effects of early social experience on physiological responsiveness. Pages 469-471 in Primatology today Elsevier New York.

Mastroeni, P., J. Chabalgoity, S. Dunstan, D. Maskell, and G. Dougan. 2001. *Salmonella*: immune responses and vaccines. The Veterinary Journal 161:132-164.

Mcfarlane, J. M., and S. E. Curtis. 1989. Multiple concurrent stressors in chicks. 3. Effects on plasma corticosterone and the heterophil: lymphocyte ratio. Poultry Science 68:522-527.

McHan, F., and E. B. Shotts. 1992. Effect of feeding selected short-chain fatty acids on the in vivo attachment of *Salmonella* Typhimurium in chick ceca. Avian Diseases:139-142.

Mead, G., and B. Adams. 1975. Some observations on the caecal micro-flora of the chick during the first two weeks of life. British Poultry Science 16:169-176.

Meddings, J. B., and M. Swain. 2000. Environmental stress–induced gastrointestinal permeability is mediated by endogenous glucocorticoids in the rat. Gastroenterology 119:1019-1028.

Meimandipour, A., M. Shuhaimi, A. Soleimani, K. Azhar, M. Hair-Bejo, B. Kabeir, A. Javanmard, O. M. Anas, and A. Yazid. 2010. Selected microbial groups and short-chain fatty acids profile in a simulated chicken cecum supplemented with two strains of *Lactobacillus*. Poultry Science 89:470-476.

Messens, W., K. Grijspeerdt, and L. Herman. 2005. Eggshell penetration by *Salmonella*: a review. World's poultry Science Journal 61:71-86.

Messens, W., K. Grijspeerdt, and L. Herman. 2006. Eggshell penetration of hen's eggs by *Salmonella enterica* serovar Enteritidis upon various storage conditions. British Poultry Science 47:554-560.

Mishra, C., and J. Lambert. 1996. Production of anti-microbial substances by probiotics. Asia Pacific Journal of Clinical Nutrition 5:20-24.

Mishu, B., P. M. Griffin, R. V. Tauxe, D. N. Cameron, R. H. Hutcheson, and W. Schaffner. 1991. *Salmonella* Enteritidis gastroenteritis transmitted by intact chicken eggs. Annals of Internal Medicine 115:190-194.

Mitchell, B., R. Buhr, M. Berrang, J. Bailey, and N. Cox. 2002. Reducing airborne pathogens, dust and *Salmonella* transmission in experimental hatching cabinets using an electrostatic space charge system. Poultry Science 81:49-55.

Mitchell, B., L. Richardson, J. Wilson, and C. Hofacre. 2004. Application of an electrostatic space charge system for dust, ammonia, and pathogen reduction in a broiler breeder house. Applied Engineering in Agriculture 20:87.

Miyamoto, T., T. Horie, E. Baba, K. Sasai, T. Fukata, and A. Arakawa. 1998. *Salmonella* penetration through eggshell associated with freshness of laid eggs and refrigeration. Journal of Food Protection® 61:350-353.

Miyamoto, T., T. Horie, T. Fujiwara, T. Fukata, K. Sasai, and E. Baba. 2000. *Lactobacillus* flora in the cloaca and vagina of hens and its inhibitory activity against *Salmonella* enteritidis in vitro. Poultry Science 79:7-11.

Moberg, G. 2000. Biological response to stress: implications for animal welfare. The biology of animal stress: basic principles and implications for animal welfare:1-21.

Moberg, G. P. 1985. Biological response to stress: key to assessment of animal well-being? Pages 27-49 in Animal StressSpringer.

Mountzouris, K., E. Dalaka, I. Palamidi, V. Paraskeuas, V. Demey, G. Theodoropoulos, and K. Fegeros. 2015. Evaluation of yeast dietary supplementation in broilers challenged or not with *Salmonella* on growth performance, cecal microbiota composition and *Salmonella* in ceca, cloacae and carcass skin. Poultry Science 94:2445-2455.

Musgrove, M., D. Jones, J. Northcutt, N. Cox, and M. Harrison. 2005. Shell rinse and shell crush methods for the recovery of aerobic microorganisms and *Enterobacteriaceae* from shell eggs. Journal of Food Protection® 68:2144-2148.

Nakamura, M., N. Nagamine, T. Takahashi, S. Suzuki, M. Kijima, Y. Tamura, and S. Sato. 1994a. Horizontal transmission of *Salmonella* Enteritidis and effect of stress on shedding in laying hens. Avian Diseases:282-288.

Nakamura, M., N. Nagamine, T. Takahashi, S. Suzuki, and S. Sato. 1994b. Evaluation of the efficacy of a bacterin against *Salmonella* Enteritidis infection and the effect of stress after vaccination. Avian Diseases:717-724.

Nakamura, M., M. Takagi, T. Takahashi, S. Suzuki, S. Sato, and K. Takehara. 1997. The effect of the flow of air on horizontal transmission of *Salmonella* Enteritidis in chickens. Avian Diseases:354-360.

Nakao, J., J. Pringle, R. Jones, B. Nix, J. Borders, G. Heseltine, T. Gomez, B. McCluskey, C. Roney, and D. Brinson. 2015. 'One Health'investigation: outbreak of human *Salmonella* Braenderup infections traced to a mail-order hatchery–United States, 2012–2013. Epidemiology and Infection 143:2178-2186.

Nandre, R., K. Matsuda, and J. Lee. 2014. Efficacy for a new live attenuated *Salmonella* Enteritidis vaccine candidate to reduce internal egg contamination. Zoonoses and public health 61:55-63.

Nayak, R., P. Kenney, J. Keswani, and C. Ritz. 2003. Isolation and characterisation of *Salmonella* in a turkey production facility. British Poultry Science 44:192-202.

Newberry, R., J. Hunt, and E. Gardiner. 1988. Influence of light intensity on behavior and performance of broiler chickens. Poultry Science 67:1020-1025.

Nielsen, B. L., M. Litherland, and F. Nøddegaard. 2003. Effects of qualitative and quantitative feed restriction on the activity of broiler chickens. Applied Animal Behaviour Science 83:309-323.

Nisbet, D. J., D. E. Corrier, and J. R. DeLoach. 1993a. Effect of mixed cecal microflora maintained in continuous culture and of dietary lactose on *Salmonella* Typhimurium colonization in broiler chicks. Avian Diseases 37:528-535.

Nisbet, D. J., D. E. Corrier, C. M. Scanlan, A. G. Hollister, R. C. Beier, and J. R. DeLoach. 1993b. Effect of a defined continuous-flow derived bacterial culture and dietary lactose on *Salmonella* Typhimurium colonization in broiler chickens. Avian Diseases:1017-1025.

Nuotio, L., C. Schneitz, U. Halonen, and E. Nurmi. 1992. Use of competitive exclusion to protect newly-hatched chicks against intestinal colonisation and invasion by *Salmonella* Enteritidis PT4. British Poultry Science 33:775-779.

Nurmi, E., L. Nuotio, and C. Schneitz. 1992. The competitive exclusion concept: development and future. International Journal of Food Microbiology 15:237-240.

Nurmi, E., and M. Rantala. 1973. New aspects of *Salmonella* infection in broiler production. Nature 241:210-211.

Ohtani, S., and S. Leeson. 2000. The effect of intermittent lighting on metabolizable energy intake and heat production of male broilers. Poultry Science 79:167-171.

Okamura, M., Y. Kamijima, T. Miyamoto, H. Tani, K. Sasai, and E. Baba. 2001. Differences among six *Salmonella* serovars in abilities to colonize reproductive organs and to contaminate eggs in laying hens. Avian Diseases:61-69.

Olanrewaju, H., J. Thaxton, W. Dozier, J. Purswell, S. Collier, and S. Branton. 2008. Interactive effects of ammonia and light intensity on hematochemical variables in broiler chickens. Poultry Science 87:1407-1414.

Olsen, A. R., and T. S. Hammack. 2000. Isolation of *Salmonella* spp. from the housefly, Musca domestica L., and the dump fly, Hydrotaea aenescens (Wiedemann) (Diptera: Muscidae), at caged-layer houses. Journal of Food Protection® 63:958-960.

Osbaldiston, G., and D. Sainsbury. 1963. Control of the environment in a poultry house. 1. The principles and practice. Veterinary Record 75:159-169; 170.

Oyofo, B., J. DeLoach, D. Corrier, J. Norman, R. Ziprin, and H. Mollenhauer. 1989. Prevention of *Salmonella* Typhimurium colonization of broilers with D-mannose. Poultry Science 68:1357-1360.

Panda, A., M. Reddy, S. R. Rao, and N. Praharaj. 2003. Production performance, serum/yolk cholesterol and immune competence of white leghorn layers as influenced by dietary supplementation with probiotic. Tropical Animal Health and Production 35:85-94.

Papezova, K., H. Havlickova, F. Sisak, V. Kummer, M. Faldyna, and I. Rychlik. 2008. Comparison of live and inactivated *Salmonella* Typhimurium vaccines containing different combinations of SPI-1 and SPI-2 antigens in poultry. Veterinarni Medicina-Praha 53:315.

Pascual, M., M. Hugas, J. I. Badiola, J. M. Monfort, and M. Garriga. 1999. *Lactobacillus salivarius* CTC2197 prevents *Salmonella* Enteritidis colonization in chickens. Applied and Environmental Microbiology 65:4981-4986.

Patrick, M. E., P. M. Adcock, T. M. Gomez, S. F. Altekruse, B. H. Holland, R. V. Tauxe, and D. L. Swerdlow. 2004. *Salmonella* Enteritidis infections, United States, 1985-1999. Emerging Infectious Diseases 10:1-7.

Patterson, J., and K. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poultry Science 82:627-631.

Paul, J., and B. Batchelor. 1988. *Salmonella* Enteritidis phage type 4 hen's eggs. The Lancet 332:1421.

Pedersen, S., and C. Pedersen. 1995. Animal activity measured by infrared detectors. Journal of Agricultural Engineering Research 61:239-246.

Pei, Y., V. R. Parreira, K. L. Roland, R. Curtiss, and J. F. Prescott. 2014. Assessment of attenuated *Salmonella* vaccine strains in controlling experimental *Salmonella* Typhimurium infection in chickens. Canadian Journal of Veterinary Research 78:23-30.

Persson, U., and S. Jendteg. 1992. The economic impact of poultry-borne salmonellosis: how much should be spent on prophylaxis? International Journal of Food Microbiology 15:207-213.

Phillips, I., M. Casewell, T. Cox, B. De Groot, C. Friis, R. Jones, C. Nightingale, R. Preston, and J. Waddell. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. Journal of Antimicrobial Chemotherapy 53:28-52.

Popoff, M. Y., J. Bockemühl, and F. W. Brenner. 2000. Supplement 1999 (no. 43) to the Kauffmann-White scheme. Research in microbiology 151:893-896.

Prayitno, D., C. Phillips, H. Omed, and D. Piggins. 1994. The effect of colour of lighting on the performance and behaviour of broilers. British Poultry Science 35:173-175.

Prayitno, D., C. Phillips, and D. Stokes. 1997. The effects of color and intensity of light on behavior and leg disorders in broiler chickens. Poultry Science 76:1674-1681.

Quastel, J. H., M. Stephenson, and M. D. Whetham. 1925. Some reactions of resting bacteria in relation to anaerobic growth. Biochemical Journal 19:304.

Quinteiro-Filho, W., A. Gomes, M. Pinheiro, A. Ribeiro, V. Ferraz-de-Paula, C. Astolfi-Ferreira, A. Ferreira, and J. Palermo-Neto. 2012a. Heat stress impairs performance and induces intestinal inflammation in broiler chickens infected with *Salmonella* Enteritidis. Avian Pathology 41:421-427.

Quinteiro-Filho, W., A. Ribeiro, V. Ferraz-de-Paula, M. Pinheiro, M. Sakai, L. Sá, A. Ferreira, and J. Palermo-Neto. 2010. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poultry Science 89:1905-1914.

Quinteiro-Filho, W. M., M. Rodrigues, A. Ribeiro, V. Ferraz-de-Paula, M. Pinheiro, L. Sa, A. Ferreira, and J. Palermo-Neto. 2012b. Acute heat stress impairs performance parameters and induces mild intestinal enteritis in broiler chickens:

Role of acute hypothalamic-pituitary-adrenal axis activation. Journal of Animal Science 90:1986-1994.

Rahimi, G., M. Rezaei, H. Hafezian, and H. Saiyahzadeh. 2005. The effect of intermittent lighting schedule on broiler performance. International Journal of Poultry Science 4:396-398.

Rahman, H., R. Reissbrodt, and H. Tschape. 2000. Effect of norepinephrine on growth of *Salmonella* and its enterotoxin production. Indian Journal of Experimental Biology 38:285-286.

Ramirez, G., L. Sarlin, D. Caldwell, C. Yezak, M. Hume, D. Corrier, and B. Hargis. 1997. Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. Poultry Science 76:654-656.

Regnier, J. A., K. W. Kelley, and C. T. Gaskins. 1980. Acute themal stressors and synthesis of antibodies in chickens. Poultry Science 59:985-990.

Renden, J., S. Bilgili, R. Lien, and S. Kincaid. 1991. Live performance and yields of broilers provided various lighting schedules. Poultry Science 70:2055-2062.

Ribeiro, A. M. L., L. K. Vogt, C. W. Canal, M. R. d. I. Cardoso, R. V. Labres, A. F. Streck, and M. C. Bessa. 2007. Effects of prebiotics and probiotics on the colonization and immune response of broiler chickens challenged with *Salmonella* Enteritidis. Revista Brasileira de Ciência Avícola 9:193-200.

Rigby, C., and J. Pettit. 1979. Some factors affecting *Salmonella* Typhimurium infection and shedding in chickens raised on litter. Avian Diseases:442-455.

Rigby, C., J. Pettit, A. Bentley, J. Spencer, M. Salomons, and H. Lior. 1982. The relationships of *Salmonellae* from infected broiler flocks, transport crates or processing plants to contamination of eviscerated carcases. Canadian Journal of Comparative Medicine 46:272.

Rikken, G., A. Kroon, and C. Van Ginkel. 1996. Transformation of (per) chlorate into chloride by a newly isolated bacterium: reduction and dismutation. Applied Microbiology and Biotechnology 45:420-426.

Roldan, M., F. Reyes, C. Moreno-Vivian, and F. Castillo. 1994. Chlorate and nitrate reduction in the phototrophic bacteria *Rhodobacter capsulatus* and *Rhodobacter sphaeroides*. Current Microbiology 29:241-245.

Roura, E., J. Homedes, and K. C. Klasing. 1992. Prevention of immunologic stress contributes to the growth-permitting ability of dietary antibiotics in chicks. The Journal of Nutrition 122:2383-2390.

Sanchez, S., C. L. Hofacre, M. D. Lee, J. J. Maurer, and M. P. Doyle. 2002. Zoonosis Update.

Sayuri Murate, L., F. Gonzales Paião, A. Maria de Almeida, A. Berchieri Junior, and M. Shimokomaki. 2014. Efficacy of prebiotics, probiotics, and synbiotics on laying hens and broilers challenged with *Salmonella* Enteritidis. The Journal of Poultry Science 52:52-56.

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. Emerging Infectious Diseases 17:7-15.

Scanes, C. G. 2016. Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. Poultry science:pew137.

Scheideler, S. E. 1990. Research Note: Effect of various light sources on broiler performance and efficiency of production under commercial conditions. Poultry Science 69:1030-1033.

Schoeni, J. L., K. A. Glass, J. L. McDermott, and A. C. Wong. 1995. Growth and penetration of *Salmonella* enteritidis, *Salmonella* heidelberg and *Salmonella* Typhimurium in eggs. International journal of food microbiology 24:385-396.

Schrezenmeir, J., and M. de Vrese. 2001. Probiotics, prebiotics, and synbiotics—approaching a definition. The American journal of clinical nutrition 73:361s-364s.

Seggie, J., and G. Brown. 1982. Profiles of hormone stress response: recruitment or pathway specificity. Pages 277-285 in Brain Peptides and HormonesRaven Press New York.

Selye, H. 1936. A syndrome produced by diverse nocuous agents. Nature 138:32.

Seo, K., P. Holt, R. Gast, and C. Hofacre. 2000. Elimination of early *Salmonella* Enteritidis infection after treatment with competitive-exclusion culture and enrofloxacin in experimentally infected chicks. Poultry Science 79:1408-1413.

Shini, S., G. Huff, A. Shini, and P. Kaiser. 2010. Understanding stress-induced immunosuppression: exploration of cytokine and chemokine gene profiles in chicken peripheral leukocytes. Poultry science 89:841-851.

Shivaprasad, H., J. Timoney, S. Morales, B. Lucio, and R. Baker. 1990. Pathogenesis of *Salmonella* Enteritidis infection in laying chickens. I. Studies on egg transmission, clinical signs, fecal shedding, and serologic responses. Avian Diseases:548-557.

Siddiqui, M. S. 1996. Chlorine-ozone interactions: formation of chlorate. Water Research 30:2160-2170.

Siegel, H. 1995. Stress, strains and resistance 1.

Siegel, H. S. 1959. The relation between crowding and weight of adrenal glands in chickens. Ecology 40:495-498.

Silva, E., G. Snoeyenbos, O. M. Weinack, and C. Smyser. 1981. Studies on the use of 9R strain of *Salmonella* Gallinarum as a vaccine in chickens. Avian diseases:38-52.

Simmons, P. Year. Effect of lighting regimes on twisted legs, feed conversion and growth of broiler-chickens. Proc. Poultry Science.

Singh, S., A. S. Yadav, S. M. Singh, and P. Bharti. 2010. Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Food Research International 43:2027-2030.

Smith, H. W. 1956. The use of live vaccines in experimental *Salmonella* Gallinarum infection in chickens with observations on their interference effect. J. Hyg 54:419-432.

Smith, M. 2003. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poultry Science 82:1580-1588.

Snoeyenbos, G., V. L. Carlson, C. Smyser, and O. M. Olesiuk. 1969. Dynamics of *Salmonella* infection in chicks reared on litter. Avian Diseases:72-83.

Soerjadi, A., J. Druitt, A. Lloyd, and R. Cumming. 1979. Effect of environmental temperature on susceptibility of young chickens to *Salmonella* Typhimurium. Australian Veterinary Journal 55:413-417.

Soleimani, A., I. Zulkifli, A. Omar, and A. Raha. 2011. Physiological responses of 3 chicken breeds to acute heat stress. Poultry Science 90:1435-1440.

Spring, P., C. Wenk, K. Dawson, and K. Newman. 2000a. Effect of mannan oligosaccharide on different cecal parameters and on cecal concentration on enteric bacteria in challenged broiler chicks. Poultry Science 79:205-211.

Spring, P., C. Wenk, K. Dawson, and K. Newman. 2000b. The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella*-challenged broiler chicks. Poultry Science 79:205-211.

Star, L., E. Decuypere, H. Parmentier, and B. Kemp. 2008. Effect of single or combined climatic and hygienic stress in four layer lines: 2. Endocrine and oxidative stress responses. Poultry Science 87:1031-1038.

Tellez, G., C. Dean, D. Corrier, J. Deloach, L. Jaeger, and B. Hargis. 1993. Effect of dietary lactose on cecal morphology, pH, organic acids, and *Salmonella* Enteritidis organ invasion in Leghorn chicks. Poultry Science 72:636-642.

Telzak, E. E., L. D. Budnick, M. S. Z. Greenberg, S. Blum, M. Shayegani, C. E. Benson, and S. Schultz. 1990. A nosocomial outbreak of *Salmonella* Enteritidis infection due to the consumption of raw eggs. New England Journal of Medicine 323:394-397.

Thaxton, P., and H. Siegel. 1970. Immunodepression in young chickens by high environmental temperature. Poultry Science 49:202-205.

Thaxton, P., R. Wyatt, and P. Hamilton. 1971. Cold stress and *Salmonella* as a cause of early mortality in chickens. Proceed Poultry Science 50:1636.

Thaxton, P., R. Wyatt, and P. Hamilton. 1974. The effect of environmental temperature on paratyphoid infection in the neonatal chicken. Poultry Science 53:88-94.

Thompson, J. L., and M. Hinton. 1997. Antibacterial activity of formic and propionic acids in the diet of hens on *Salmonellas* in the crop. British poultry science 38:59-65.

Traub-Dargatz, J. L., S. R. Ladely, D. A. Dargatz, and P. J. Fedorka-Cray. 2006. Impact of heat stress on the fecal shedding patterns of *Salmonella enterica* Typhimurium DT104 and *Salmonella enterica* Infantis by 5-week-old male broilers. Foodbourne Pathogens and Disease 3:178-183.

Tsai, C.-C., H.-Y. Hsih, H.-H. Chiu, Y.-Y. Lai, J.-H. Liu, B. Yu, and H.-Y. Tsen. 2005. Antagonistic activity against *Salmonella* infection in vitro and in vivo for two *Lactobacillus* strains from swine and poultry. International Journal of Food Microbiology 102:185-194.

United States Department of Agriculture 2015a. Food Availability (Per Capital) Data system. http://www.ers.usda.gov/data-products/food-availability-(per-capita)-data-system/.aspx.

United States Department of Agriculture 2015b. Foodborne illnesses caused by *Salmonella* cost the U.S. an estimated $3.7 billion annually. http://www.ers.usda.gov/data-products/chart-gallery/detail.aspx?chartId=50500.

Van Coillie, E., J. Goris, I. Cleenwerck, K. Grijspeerdt, N. Botteldoorn, F. Van Immerseel, J. De Buck, M. Vancanneyt, J. Swings, and L. Herman. 2007. Identification of *Lactobacilli* isolated from the cloaca and vagina of laying hens and characterization for potential use as probiotics to control *Salmonella* Enteritidis. Journal of Applied Microbiology 102:1095-1106.

Van der Wielen, P. W., S. Biesterveld, S. Notermans, H. Hofstra, B. A. Urlings, and F. Van Knapen. 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Applied and Environmental Microbiology 66:2536-2540.

Van Ginkel, C., C. Plugge, and C. Stroo. 1995. Reduction of chlorate with various energy substrates and inocula under anaerobic conditions. Chemosphere 31:4057-4066.

Van Immerseel, F. 2007. Preventing *Salmonella* infections by rationally designed feed additives: the use of organic acids. Lohmann Inf 42:10-17.

Van Immerseel, F., F. Boyen, I. Gantois, L. Timbermont, L. Bohez, F. Pasmans, F. Haesebrouck, and R. Ducatelle. 2005. Supplementation of coated butyric acid in the feed reduces colonization and shedding of *Salmonella* in poultry. Poultry Science 84:1851-1856.

Van Immerseel, F., J. De Buck, F. Boyen, L. Bohez, F. Pasmans, J. Volf, M. Sevcik, I. Rychlik, F. Haesebrouck, and R. Ducatelle. 2004a. Medium-chain fatty acids decrease colonization and invasion through hilA suppression shortly after infection of chickens with *Salmonella enterica* serovar Enteritidis. Applied and Environmental Microbiology 70:3582-3587.

Van Immerseel, F., V. Fievez, J. De Buck, F. Pasmans, A. Martel, F. Haesebrouck, and R. Ducatelle. 2004b. Microencapsulated short-chain fatty acids in feed modify colonization and invasion early after infection with *Salmonella* Enteritidis in young chickens. Poultry Science 83:69-74.

Vicente, J. L., A. Torres-Rodriguez, S. E. Higgins, C. Pixley, G. Tellez, A. M. Donoghue, and B. M. Hargis. 2008. Effect of a selected *Lactobacillus* spp.-based probiotic on *Salmonella enterica* serovar Enteritidis-infected broiler chicks. Avian Diseases 52:143-146.

Vilà, B., A. Fontgibell, I. Badiola, E. Esteve-Garcia, G. Jiménez, M. Castillo, and J. Brufau. 2009. Reduction of *Salmonella* enterica var. Enteritidis colonization and invasion by Bacillus cereus var. toyoi inclusion in poultry feeds. Poultry Science 88:975-979.

Volkova, V. V., J. A. Byrd, S. A. Hubbard, D. Magee, R. H. Bailey, and R. W. Wills. 2010. Lighting during grow-out and *Salmonella* in broiler flocks. Acta Veterinaria Scandinavica 52:1.

Waltman, W., and R. Gast. 2008. Salmonellosis. Pages 3 – 9: In A Laboratory manual for the isolation and identification of avian pathogens. D. Swayne, Glisson, JR,, M. Jackwood, Pearson, JE,, and W. Reed, Jackwood, MW, Woolcock, PR, eds. American Association of Avian Pathologists, Athens, GA.

Washburn, K. 1985. Breeding of poultry in hot and cold environments. Stress physiology in livestock. Volume III. Poultry.:111-122.

Watkin, B. A., and B. F. Miller. 1983. Competitive gut exclusion of avian pathogens by *Lactobacillus acidophilus* in gnotobiotic chicks. Poultry Science 62:1772-1779.

Wilson, J., W. Weaver, W. Beane, and J. Cherry. 1984. Effects of light and feeding space on leg abnormalities in broilers. Poultry science 63:565-567.

Wilson, L. M., and A. L. Baldwin. 1999. Environmental stress causes mast cell degranulation, endothelial and epithelial changes, and edema in the rat intestinal mucosa. Microcirculation 6:189-198.

Wilson, S., and F. Cunningham. 1980. Effect of increasing day length and intermittent lighting schedules in the domestic hen on plasma concentrations of luteinizing hormone (LH) and the LH response to exogenous progesterone. General and Comparative Endocrinology 41:546-553.

Withanage, G., P. Wigley, P. Kaiser, P. Mastroeni, H. Brooks, C. Powers, R. Beal, P. Barrow, D. Maskell, and I. McConnell. 2005. Cytokine and chemokine responses associated with clearance of a primary *Salmonella* enterica serovar Typhimurium infection in the chicken and in protective immunity to rechallenge. Infection and Immunity 73:5173-5182.

Witte, W. 2000. Ecological impact of antibiotic use in animals on different complex microflora: environment. International journal of antimicrobial agents 14:321-325.

Woodward, M. J., G. Gettinby, M. Breslin, J. Corkish, and S. Houghton. 2002. The efficacy of Salenvac, a *Salmonella* enterica subsp. Enterica serotype Enteritidis iron-restricted bacterin vaccine, in laying chickens. Avian Pathology 31:383-392.

Wray, C., R. Davies, and S. Evans. Year. *Salmonella* infection in poultry: the production environment. Proc. Poultry Meat Science, Poultry Science Symposium Series Volume Twenty-Five. CABI Publishing.

Xie, D., Z. Wang, Y. Dong, J. Cao, J. Wang, J. Chen, and Y. Chen. 2008. Effects of monochromatic light on immune response of broilers. Poultry Science 87:1535-1539.

Xie, J., L. Tang, L. Lu, L. Zhang, X. Lin, H.-C. Liu, J. Odle, and X. Luo. 2015. Effects of acute and chronic heat stress on plasma metabolites, hormones and oxidant status in restrictedly fed broiler breeders. Poultry Science 94:1635-1644.

Yhiler, N. Y., and B. E. Bassey. 2015. Critical segments in the dissemination and transmission of *Salmonella* Species from Poultry Production in Calabar, Nigeria. Science Journal of Public Health 3:168-174.

Yildirim, Y., Z. Gonulalan, S. Pamuk, and N. Ertas. 2011. Incidence and antibiotic resistance of *Salmonella* spp. on raw chicken carcasses. Food Research International 44:725-728.

Zahraa, H., and A. Ghamdi. 2008. Effects of commutative heat stress on immunoresponses in broiler chickens reared in closed system. International Journal of Poultry Science 7:964-968.

Zarnitsyna, V. I., and C. Zhu. 2011. Adhesion frequency assay for in situ kinetics analysis of cross-junctional molecular interactions at the cell-cell interface. Journal of Visualized Experiments:e3519-e3519.

Zhang-Barber, L., A. Turner, and P. Barrow. 1999. Vaccination for control of *Salmonella* in poultry. Vaccine 17:2538-2545.

Zhang, G., L. Ma, and M. P. Doyle. 2007. *Salmonellae* reduction in poultry by competitive exclusion bacteria *Lactobacillus salivarius* and *Streptococcus cristatus*. Journal of Food Protection® 70:874-878.

Zorrilla, E. P., L. Luborsky, J. R. McKay, R. Rosenthal, A. Houldin, A. Tax, R. McCorkle, D. A. Seligman, and K. Schmidt. 2001. The relationship of depression and stressors to immunological assays: a meta-analytic review. Brain, Behavior, and Immunity 15:199-226.