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Salmonella typhimurium infection in broilers and its effects on gastrointestinal health and performance

By

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I, Taryn Lee Halsey declare that the thesis/dissertation, which I hereby submit for the degree M.Sc. (Agric) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE:……..

DATE:……

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Dedicated in loving memory to my mother Merle Catherine Halsey

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Abstract

Salmonella typhimurium (ST) infection not only causes salmonellosis in humans, but also can result in great economic losses in the typically narrow-margin, high-volume broiler business due to reduced growth rates and mortalities. Over the last decade, the use of antibiotics and attenuated vaccines to restrain or prevent bacterial infections in domestic animals has been criticised because of the possible development of antibiotic resistance and the potential dangers of residual antibiotics and vaccines in animal-derived food products for human consumption. For these reasons, many countries have begun phasing out growth promoting antibiotics in broiler diets. It is therefore essential for the poultry production industry to develop feed additives and processing techniques as alternatives for sub-therapeutic dietary supplementation of antibiotics. However, innovative research is needed to evaluate the efficacy of new and existing alternative products.

The general aim of this trial was to determine the effects of *Salmonella typhimurium* colonisation of the gastrointestinal tract of broiler chicks on gastrointestinal health and production performance. The effect of Zinc-Bacitracin (Zn-BC), a commonly used antibiotic growth promoter in the poultry industry, on Salmonella colonisation was also measured.

A pilot trial was first conducted to determine the level of *Salmonella typhimurium* required to infect broiler chicks, and the necessity of administering an immunosuppressive agent in order to obtain infection. The main trial followed to determine the effects of *Salmonella typhimurium* on gastrointestinal health and function. The ultimate aim of the study was to obtain baseline values of various parameters that could be used in future trials for the evaluation of antibiotic alternative products. The results obtained from the pilot trial showed that it was not necessary to administer cyclophosphamide as the *Salmonella typhimurium* proved to be highly virulent. The cloacal swabs taken in the second trial showed that the use of Zn-BC as an antibiotic did not inhibit Salmonella colonisation in the challenged birds. The inclusion of Zn-BC in this trial inhibited the growth of the gut microflora allowing the Salmonella to proliferate in the body of the chicken, which lead to the conclusion that the routine inclusion of Zn-BC at sub-clinical levels as a growth promoter may be detrimental when the bird gets exposed to Gram(-) bacteria, such as Salmonella.

In both of the trials, Salmonella challenge resulted in enlargement of the organs with a consequent increase in the organ weights. In the pilot trial there was a significant difference ($P<0.0033$) of the control weights for the duodenum, ileum, caeca and liver and those of the Salmonella infected birds. Control birds that did not receive CY had duodenum weights of 1.00 (±0.236) while the birds infected with 1×10^8 CFU/mL had weights of 1.99 (±0.310), while the control birds that did receive CY had duodenum weights of 0.98 (±0.244) with the Salmonella infected birds having weights of between 1.79 (±0.299) and 2.13 (±0.006). Significant results ($P<0.016$) in the main trial were found to occur predominantly at 7 days of

age for the duodenum, jejenum, ileum and caeca weights. Control birds in the group that did receive antibiotics had 7 day duodenum weights of 1.80 (±0.301) compared to the Salmonella infected bird which had weights of between 2.33 (±0.376) and 2.51 (±0.424).

In general Salmonella did not affect the growth and performance of the challenged birds. Birds challenged with Salmonella showed a tendency to have enlarged livers, possibly due to hepatic damage. In the main trial there was a significant difference ($P<0.016$) in liver weights at 28 days of age between the control and Salmonella infected groups regardless of whether the birds received antibiotics or not. The control birds that received antibiotics had liver weights of 3.24 (±0.234) while the birds infected with the higher level of Salmonella had weights of 3.86 (±0.542). This finding, together with the noticeable, although mainly insignificant, trend of decreased serum albumin levels and increased serum globulin and total serum protein levels noted in infected birds can be used in conjunction to measure the effect of ST on liver damage.

Salmonella colonisation resulted in an increase in the severity of lesions seen in the gastrointestinal tract ($P<0.0016$). Histopathology results proved to be inconsistent and did not provide any conclusive evidence on the effect of Salmonella on the organs. Villi measurements taken in the second trial showed that Salmonella significantly ($P<0.016$) shortened the length of the villi in the duodenum and jejenum of challenged birds when measured at 28 days of age. Control birds had duodenum villi length of 662.5 (±56.79) while those birds infected with Salmonella had lengths of between 558.9 (±77.74) and 537.0 (±51.66). There was a significant difference in the duodenum villi length regardless of antibiotic inclusion into the diet. In the birds that did receive antibiotics, there was a significant difference ($P<0.016$) in the jejenum villi length with the control birds having the longest villi 725.7 (±90.92) while the birds infected with the higher level of Salmonella having the shortest villi 557.2 (±124.5). It would appear that using all of the information and results obtained for liver weights, broiler performance, serum biochemical level, lesion scoring, histopathology and villous morphological measurements should be used in conjunction with one another to measure the effect of Salmonella on the broiler chicken.

The results obtained in this trial clearly show just how significant a problem Salmonella infection can be in the poultry industry due to seemingly healthy adult birds displaying little or no systemic disease being non-symptomatic carriers. Many of the Para-typhoid salmonellae do not always produce clinical signs in chicks, and their presence in the poultry industry may go unrecognised for this reason.

Abbreviations

AIDS: Acquired immune Deficiency Syndrome

ST: *Salmonella typhimurium*

SE: *Salmonella enteritidis*

USDA: United States Department of Agriculture

spp: Species

SPI: Salmonella Pathogenicity Island

PT: Paratyphoid

TTSS: Type III secretion system

TLR: Toll-like receptor

Ig: Immunoglobulin

AGP: Antibiotic Growth promoter

BC: Bacitracin

Zn-BC: Zinc-Bacitracin

CE: Competitive Exclusion

MALT: Mucosa associated lymphoid tissue

GALT: Gut-associated lymphoid tissue

CT: Caecal tonsils

PP: Peyer's patches

IEL: Intraepithelial lymphocytes

mg: Milligram

mL: Millilitre

CY: Cyclophosphamide

g: Gram

Kg: Kilogram

GIT: Gastrointestinal tract

IM: Intramuscular

CFU: Colony forming units

TSP: Total serum protein

AST: Aspartate aminotransferase

NA: Nothing abnormal

NR: Nothing remarkable

ADG: Average daily gain

FI: Feed intake

FCR: Feed conversion ratio

BW: Body weight

AB: Antibiotic

NAB: No antibiotics

MFN: Multifocal necrosis

MFJN: Multifocal junctional necrosis

ROS: Reactive oxygen species

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Chapter 1
Introduction

Salmonella is a leading cause of bacterial food-borne disease outbreaks in developed countries and is a public health concern in developing countries, with diarrhoea killing up to three million children each year in developing countries. While *Salmonella typhimurium* will mainly cause food poisoning in humans, infections can lead to bacteraemia, particularly in immune-compromised people, such as AIDS patients. *S. typhimurium* is also the most common serovar causing cardiovascular, bone and joint infections in humans (Amy *et al*., 2004; Bohez *et al.*, 2006; Lan *et al*., 2007). During the past 10-15 years, many countries have experienced a steady to dramatic increase in the incidence of clinical infection involving Salmonella. The increase has been so widespread, and in some cases so dramatic, as to be described as a new pandemic (Cox, 1995; Fernández *et al*., 2001; Amy *et al*., 2004; Bohez *et al.*, 2006).

Many cases of human salmonellosis are attributed to the consumption of infected poultry meat and eggs; the vast majority of cases being the result of *S. enterica* serovar Enteritidis or serovar Typhimurium (Beal *et al.,* 2004; Lan *et al.,* 2007). The elimination of this source of human contamination is hindered by the fact that poultry can be asymptomatic carriers of Salmonella showing no signs of infection (Fernández *et al.,* 2001; Amy *et al.,* 2004).

Chickens are reared under intensive conditions that are conducive to infection by opportunistic pathogens. A major problem faced by the poultry industry is loss of productivity due to disease; therefore, considerable resources are required in order to maintain the health status of these animals (Lowenthal *et al*., 2000). Salmonella can be widespread in the production and processing environments (Tamblyn & Conner, 1997) resulting in the entry of Salmonella into the human food chain (Turner *et al*., 1998). An eradication program for Salmonella infections would likely cost the consumer far more than could be justified by the benefits derived from such a program (Calnek *et al*., 1991).

Salmonella is capable of causing highly virulent systemic disease in young chicks of less than 3 days of age, while Salmonella infection rarely causes clinical disease in chicks over 3 weeks of age (Calnek *et al*., 1991; Beal *et al*., 2006) and even then mortality in the older chicks may be high only when other adverse conditions are present (Calnek *et al*., 1991). Salmonella can cause persistent colonisation of the gastrointestinal tract, with older birds becoming asymptomatic carriers of the organism (Beal *et al.,* 2004; Lan *et al.,* 2007). Apart from causing salmonellosis in humans, these infections are also responsible for reduced growth rates, mortalities and consequently great economic losses in the poultry industry.

Antimicrobials were introduced into human chemotherapy in the 1940s, and soon after they were introduced into veterinary practice (Witte, 2000), finding widespread use in the livestock industry as therapeutic agents and growth promoters for the treatment and prevention of bacterial infections (Joerger, 2003; Skjolaas *et al.,* 2007). This resulted in the frequent and long term exposure of a large number of animals to sub-therapeutic concentrations of antimicrobials (Dierick *et al*., 2002; El-Abasy *et al*., 2004; De Oliveira *et a*l., 2005). The use of antibiotics as growth promoters have subsequently come under increasing scrutiny by some scientists, consumers and government regulators because of the potential development of antibiotic-resistant human pathogenic bacteria after prolonged use (Dahiya *et al*., 2006). Consequently, numerous alternative methods for the control of enteric bacterial pathogens in animals and humans have been investigated (Montagne *et al.*, 2003). The exclusion of antibiotic growth promoters (AGPs) from the livestock industry can, however, have negative effects, such as increased production costs and thus end-product prices. Therefore the policy decision must involve a trade-off between the public health and economic benefits (Kelly *et al*., 2004).

It is essential for the poultry production industry to develop new feed additives and processing techniques as substitutes for sub-therapeutic dietary supplementation of antibiotics. Finding replacements for AGPs will likely involve the use of multiple products in the diet. It is unlikely that a single replacement will be found that will prove to be economically viable (Dibner & Richards, 2005). Recently, many alternatives have been suggested to alter the microflora for the benefit of animal health and production and combat against pathogenic organisms such as Salmonella. Examples of such alternative products include exogenous enzymes, probiotics, fermentable carbohydrates, zinc and dietary acidifiers (Bogaard & Stobberingh 2000; Berndt & Methner, 2001; Joerger, 2003; Collignon, 2004; Dibner & Richards, 2005). Many of these products have not been tested or proved effective against Salmonella or other pathogenic microorganisms. It is difficult to quantify the efficacy of such products under experimental conditions, complicating its assessment.

The general aim of this trial was to determine the effects of *Salmonella typhimurium* colonisation of the gastrointestinal tract of broiler chicks on gastrointestinal health and production performance. In order to determine these effects, intestinal damage, organ weights, serum biochemical profile and performance parameters were measured. Histopathological sampling was also done on various organs. The effect of Zinc-Bacitracin, a commonly used AGP in the poultry industry, on Salmonella colonisation was also measured. The ultimate aim of the study was to obtain baseline values of various parameters that could be used in future trials for the evaluation of antibiotic alternative products.

The null hypothesis was that Salmonella colonisation of the gastrointestinal tract of broiler chicks does not have any quantifiable effects on the birds and therefore evaluating alternative

products to control Salmonella infection is impossible. The alternative hypothesis was that colonisation does have quantifiable effects therefore making evaluation of alternative products possible.

CHAPTER 2

Literature Review

Poultry farming is one of the most intensive forms of livestock farming, with the conditions under which chickens are reared being favourable for infection by opportunistic pathogens. The loss of productivity caused by disease is one of the major problems facing intensive livestock industries, and considerable resources are required to maintain the health of these animals (Lowenthal *et al*., 2000).

The poultry industry contributes approximately 16% of the total gross value of agriculture in South Africa. The broiler industry currently produces on average 13.8 million broilers per week, growing steadily from 1990 when only 7.6 million broilers per week were produced. The domestic demand for poultry meat is estimated to be rising by about 7% per annum, surpassing the performance of any other proteins on the market. The steady growth of South Africa's economy will increase the demand for poultry meat. It is expected that the growth in poultry meat consumption will continue, with imports increasing substantially over the next few years. Imports currently represent approximately 10% of the total value of the poultry market in South Africa or more than 20% of production (Republic of South Africa, Poultry and Products Voluntary Report 2007, GAIN Report Number: SF7042, 2007).

1. Salmonella

1.1 Enterobacteriaceae

The family Enterobacteriaceae consists of Gram-negative aerobic or facultatively anaerobic, asporogenous rod-shaped bacteria. The family consists of a great number of antigenically related and biochemically comparable bacteria which includes *Salmonella, Escherichia, Shigella, Citrobacter, Klebsiella* and *Proteus*. They are prevalent in the environment with most largely being intestinal parasites (Jordan & Pattison, 1996).

1.2 Salmonellosis

Avian salmonellosis can be classified as acute or chronic avian diseases caused by any of the members of the bacterial genus *Salmonella*. The genus *Salmonella* was named after the late USDA veterinarian, Daniel E. Salmon (Calnek *et al*., 1991). The classification of Salmonellae has been controversial for many years. According to the latest nomenclature, the genus *Salmonella* consists of only two species: *S. enterica* and *S. bongori.* The species *S. enterica* is further divided into six subspecies which are listed in Table 2.1 (Jordan & Pattison, 1996).

Table 2.1 Subspecies of *Salmonella enterica* (Jordan & Pattison, 1996)

Subspecies I	*enterica*
Subspecies II	*salamae*
Subspecies IIIa	*arizonae*
Subspecies IIIb	*diarizonae*
Subspecies IV	*houtenae*
Subspecies VI	*indica*

About 99% of all Salmonella infections in warm-blooded animals are caused by *S. enterica* subspecies I. The subspecies I strains are further differentiated as 'serovars' as shown in Fig. 2.1 (Ehrbar & Hardt, 2005).

The course of the disease is dependant on the type of *Salmonella* serovar as well as on the infected host. Some *S.enterica* subspecies I serovars are limited to a particular host. For example, *S. enterica* subspecies I serovar Typhi (*S.typhi*) strains only infect humans causing a life threatening systemic infection called typhoid fever (Jongerius-Gortemaker *et* al., 2002; Ehrbar & Hardt, 2005; Trebichavsky *et al*., 2006; Perron *et al*., 2007). Other serovars, like *S. enterica* subspecies I serovar Typhimurium (*S. typhimurium*) have a broad host range and are commonly found in livestock (chicken, swine, and cattle) (Hinton *et* al., 1990; Calnek *et* al., 1991; Ehrbar & Hardt, 2005; Okamura *et al*., 2007).

Turner *et al.* (1998) reported that more than 2000 serotypes had been identified, while Jordan & Pattison (1996) reported that, at the time, 2296 different Salmonella serovars had been identified. By 2007 even more than 2500 serovars of *Salmonella enterica* had been identified (Butaye *et al*., 2006; Perron *et al*., 2006; Vo *et al*., 2006; Perron *et al*., 2007) and while some state that few appear to cause foodborne illnesses (Perron *et al*., 2006; Perron *et al*., 2007) and little or no systemic disease in healthy adult animals (Turner *et al*., 1998), others claim that the majority of the serovars are capable of causing infections in adult animals (Vo *et al*., 2006). Furthermore, there are those who feel that although all serotypes may be considered as potential human pathogens, the majority of infections are caused by a very limited number of serotypes (Butaye *et al*., 2006). This contrasts with Jordan & Pattison (1996) who reported that all members of the Salmonella species are considered to be potentially pathogenic, but that different serovars differ widely in their host range and the pathogenic syndromes that they produce.

In veterinary literature, a distinction is usually made between infections caused by serovars of *S. pullorum* (pullorum disease), *S. gallinarum* (fowl typhoid), the arizonae group of Salmonellae (arizonosis) and the remainder of the Salmonellae (salmonellosis, paratyphoid infection) (Jordan & Pattison, 1996).

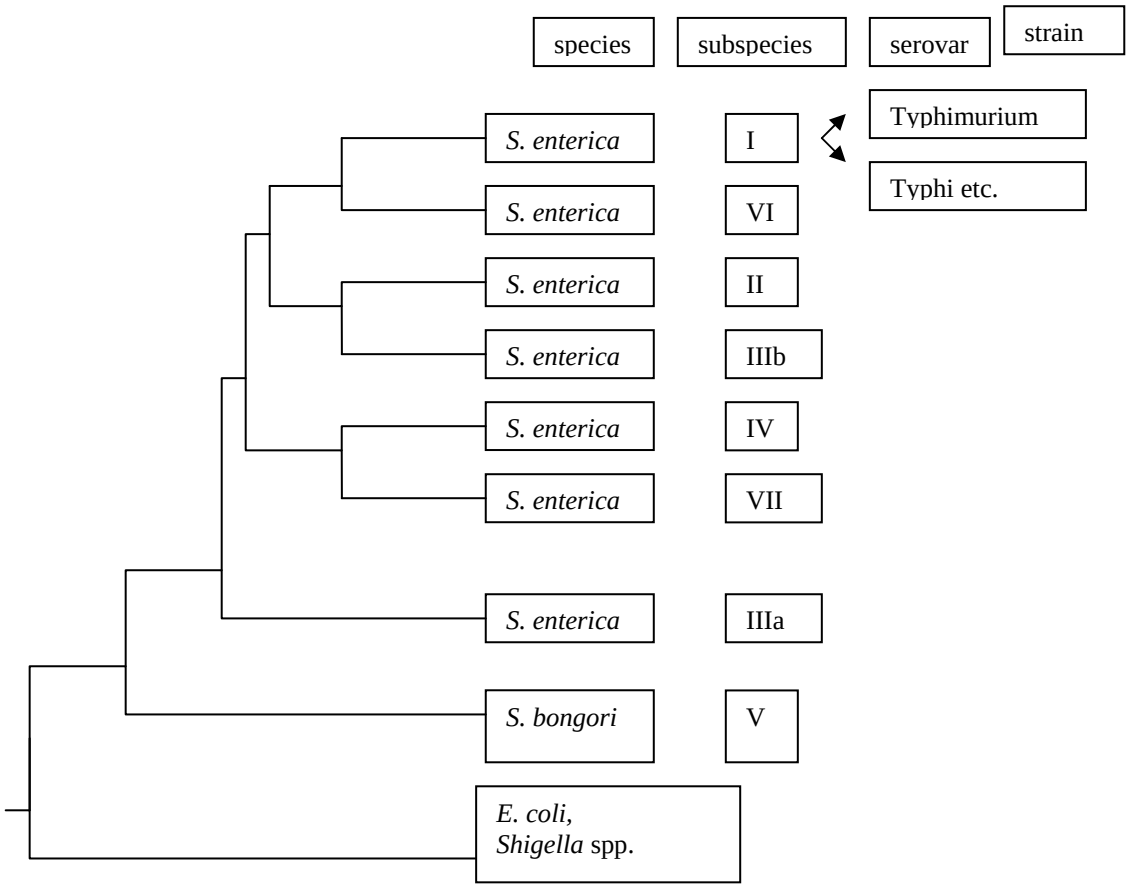

Fig. 2.1 Dendrogram showing the phylogenetic relationship among Salmonella subspecies (Ehrbar & Hardt, 2005)

Paratyphoid (PT) infections of poultry exist throughout the world, with several different serovars having been identified in domestic poultry. One serovar may be found to predominate for years before being replaced by another serovar (Jordan & Pattison, 1996), with formerly rare serotypes being found to become increasingly common in one region or country (Calnek *et al*., 1991).

PT infections are, from an economic viewpoint, amongst the bacterial diseases most significant to the hatching industry, resulting in high mortality rates among all types of young poultry (Calnek *et al*., 1991). According to Cason *et al. (*1994), many of the PT Salmonellae do not always produce clinical signs in chicks, and their presence in the poultry industry may go unrecognised for this reason.

1.3 Causal organism

Salmonella are Gram-negative (Jordan & Pattison, 1996; Yeh *et al*., 2002; Ehrbar & Hardt, 2005; Guntupalli *et al*., 2007) non-sporing rods (2-4 x 0.5µm) that do not have capsules (Jordan & Pattison, 1996). They are facultative intracellular pathogens (Yeh *et al*., 2002;

Trebichavsky *et al*., 2006) and all except *S. pullorum* and *S. gallinarum* are usually motile with long flagellae. They grow well on ordinary media and on agar, forming large, thick, greyish-white, dome-shaped colonies. All Salmonella can survive for several months away from the host (Jordan & Pattison, 1996).

According to Yeh *et al. (*2002), the gastrointestinal tract (GIT) of warm- and cold-blooded animals serves as the main reservoir for Salmonella species. The GIT is invaded through the mucous membranes and spread by both faecal and oral transmission. Salmonella are pathogenic bacteria which can cause diseases ranging from mild, self-limiting enterocolitis (food poisoning) to systemic infections (typhoid fever) (Ehrbar & Hardt, 2005; Trebichavsky *et al*., 2006), and acute enteritis (Trebichavsky *et al*., 2006).

According to Berndt & Methner (2001; 2004), Hinton (1999), Kang & Fung (2000) and Lan *et al. (*2007), *S. typhimurium* (ST) and *S. enteritidis* (SE) are currently the serovars of Salmonella most frequently linked with human food poisoning, which was confirmed by Jongerius-Gortemaker *et al. (*2002), who reported that these serovars represented 74% of all Salmonella isolates from human sources, while Leon-Velarde *et al. (*2004), Perron *et al. (*2006; 2007) and Yang *et al. (*2004) reported that ST is responsible for 40-70% of all human salmonellosis cases.

1.4 Incidence, distribution, transmission and economic importance

1.4.1 Foodborne infections: Salmonella is an important pathogenic microorganism for the food and livestock industry, causing foodborne outbreaks and enteric infection in both humans and animals (Jordan & Pattison, 1996; Ricke *et al*., 1997; Tan *et al*., 1997a ; Hinton, 1999; Jongerius-Gortemaker *et al*., 2002; Lailler *et al*., 2002; De Siqueira *et al*., 2003; Reis *et al*., 2003; Amy *et al*., 2004; Yang *et al*., 2004; Steingroewer *et al*., 2007) resulting in mild to severe clinical effects (Jongerius-Gortemaker *et al*., 2002).

According to Berndt & Methner (2001; 2004), salmonellosis belongs to the most important foodborne zoonoses throughout the world, with foodborne pathogens causing food contamination at every stage of production, processing, and distribution (Guntupalli *et al*., 2007).

1.4.2 Distribution: Infections caused by motile Salmonellae have been documented in poultry from as early as 1899 (Jordan & Pattison, 1996). Salmonella is widely distributed in nature, colonising a range of animal hosts including mammals, amphibians, reptiles, birds and insects (Butaye *et al*., 2006), and has been reported in a large number of wild bird species (Jordan & Pattison, 1996). Infections are generally sub-clinical and are commonly found in domestic poultry throughout the world (Calnek *et al*., 1991; Jordan & Pattison, 1996; Butaye *et al*., 2006).

Salmonella typhimurium has become a major cause of enteric infections in several countries including Britain, USA, Canada (Beaudin *et al*., 2002; Butaye *et al*., 2006; Lan *et al*., 2007) and Australia (Lan *et al*., 2007) with the number of reported cases of salmonellosis increasing about threefold in the United States during the past 20 years (Yeh *et al*., 2002).

1.4.3 Economic importance: The steady and dramatic growth seen in the poultry industry over the past decade, as well as the widespread occurrence of avian salmonellosis in the industry has resulted in salmonellosis becoming the most economically important egg-borne bacterial diseases of poultry (Calnek *et al*., 1991; Lowenthal *et al*., 2000). Nationwide programs to control salmonellosis infections have been met with several obstacles, as these infections recognise no international boundaries and have few host barriers (Calnek *et al*., 1991; Jordan & Pattison, 1996).

Due to its chronic nature and the difficulty of its eradication, PT has the ability to close down breeding operations in which large amounts of capital may have been invested (Calnek *et al*., 1991). Estimates of the annual costs of this infection in several countries have also proved that it is a serious economic problem for these societies (Hoszowski & Truszcyński, 1997).

The major sources of Salmonella in the poultry production chain include infected breeders, contaminated feed (Hinton *et al*., 1990; Hoszowski & Truszcyński, 1997), faecal material in the litter (Hoszowski & Truszcyński, 1997; Ricke *et al*., 1997) the environment where the animals are reared (Hinton *et al*., 1990; Hinton *et al*., 1999; Hoszowski & Truszcyński, 1997), intestinal colonisation (Ricke *et al*., 1997) as well as slaughtering and processing plants (Hinton *et al*., 1990; 1999). Effective means of protecting poultry flocks against infection are needed to decrease the transmission of Salmonella from poultry products to humans (Hoszowski & Truszcyński, 1997) although it is possible for humans to contract the infection via non-foodborne routes such as during contact with animals, contaminated water, or the environment (Vo *et al*., 2006).

According to Vo *et al.* (2006) the extensive distribution of food is a “global challenge” for Salmonella control programs. Increased travel and global trade has resulted in outbreaks occurring more frequently, with contaminated food produced in one country able to cause illness in another. This clearly demonstrates the immense importance of national control programs with sensitive and precise detection methods for the routine screening of Salmonella contamination (Hoszowski & Truszcyński, 1997; Yeh *et al*., 2002; Vo *et al*., 2006).

Tan *et al*. (1997a) states that the requirement is for “protection against perseverance of Salmonella in chickens rather than against the disease itself”. By decreasing the incidence of Salmonella infection in poultry the risk of poultry products sold for human consumption being contaminated with Salmonella would be drastically reduced (Hoszowski & Truszcyński, 1997;

Tan *et al*., 1997a; Yeh *et al*., 2002; Vo *et al*., 2006).

1.4.4 Public concern and eradication: Public demand for high quality foods has encouraged national interest in avian salmonellosis, resulting in the poultry industry having to take measures to eradicate sources of Salmonellae at all levels of production (Calnek *et al*., 1991; Jordan & Pattison, 1996). This is hindered by the fact that the protocol for the isolation and identification of Salmonella organisms can take up to 3-6 days or more to yield indisputable results (De Siqueira *et al*., 2003).

According to Calnek *et* al. (1991) Salmonella eradication programmes would in all probability end up costing the consumer far more than could ever be justified by the benefits derived from such a program. However, the increasing occurrence of multidrug-resistant strains of Salmonella (Butaye *et al*., 2006; Perron *et al*., 2006; Perron *et al*., 2007; Shahada *et al*., 2007) justifies the continuous worldwide surveillance of this organism (Butaye *et al*., 2006). According to Lesne *et al.* (2000) such efforts at surveillance have become increasingly common, and have been accomplished through the progressive application of new technology developed in the course of research (Calnek *et al*., 1991).

1.4.5 Carriers and transmission: Salmonellae are able to colonise the alimentary tracts of livestock, birds, cattle, and rodents (Turner *et* al., 1998; Oh *et al*., 2004). Salmonellae can occur naturally in the intestines of chickens and can therefore become established in production and processing environments (Tamblyn & Conner, 1997; Turner *et al*., 1998) resulting in the entry of Salmonellae into the human food chain (Turner *et al*., 1998).

Poultry contaminated with Salmonella has been implicated as one of the main sources of food associated outbreaks of human salmonellosis (Hinton *et al*., 1990; Calnek *et al*., 1991; Ricke *et al*., 1997; Tamblyn & Conner, 1997; Turner *et al*., 1998; Kwon & Ricke, 1999; Berndt & Methner, 2001; Amy *et al*., 2004; Berndt & Methner, 2004; Oh *et al*., 2004; Lan *et al*., 2007; Oscar, 2007) which is a major concern for the poultry industry, as consumers frequently distinguish chicken as a potential health risk due to its association with Salmonellae (Tamblyn & Conner, 1997).

According to Butaye *et al.* (2006) and Ehrbar & Hardt (2005), animals produced for food are the main reservoir for human infections in industrialised countries, with the majority of the illness being linked to contaminated meat and egg products. Contaminated poultry products (Shamsuzzam *et al*., 1989; Jordan & Pattison, 1996; Hoszowski & Truszcyński, 1997; Tan *et al*., 1997a ; Chriél *et al*., 1999; Hinton *et al*., 1999; Nutt *et al*., 2003; Beal *et al*., 2004; Beal *et al*., 2006; Butaye *et al*., 2006; Perron *et al*., 2006; Vo *et al*., 2006; Guntupalli *et al*., 2007; Perron *et al*., 2007; Steingroewer *et al*., 2007), egg and egg products (Shamsuzzam *et al*., 1989; Nutt *et al*., 2003; Beal *et al*., 2004; Oh *et al*., 2004; Ehrbar & Hardt, 2005; Beal *et al*.,

2006; Steingroewer *et al*., 2007), dairy products (Shamsuzzam *et al*., 1989; Oh *et al*., 2004; Steingroewer *et al*., 2007), shellfish (Oh *et al*., 2004), beef (Hinton *et al*., 1999; Lan *et al*., 2007) and pork (Hinton *et al*., 1999) are all possible sources of contamination.

1.4.6 Fruit and vegetables: While contaminated foods are often of animal origin, all foods including fruits and vegetables can serve as a source of contamination (Leon-Velarde *et al*., 2004; Steingroewer *et al*., 2007). According to Nutt *et al.* (2003), foodborne illnesses associated with fresh produce have been identified in a wide range of fruit and vegetables, as well as in unpasteurised fruit juices. With people starting to consume more fruits and vegetables for health and nutritional benefits this will become an increasingly important issue.

Nutt *et al.* (2003) reported that contamination in fruits and vegetables can originate from numerous sources, including the soil, insects, animals or even humans. The use of polluted irrigation water as well as the use of raw animal manure as fertiliser will increase the risk of enteric pathogens such as Salmonella contaminating fruits and vegetables.

1.5 Symptoms in infected humans: Salmonella infections range in severity (Jones *et al*., 1993) causing mild to severe clinical effects (Jongerius-Gortemaker *et al*., 2002). The symptoms of salmonellosis occur 12-72 hours after infection, and will usually last 4-7 days (Oh *et al*., 2004; Steingroewer *et al*., 2007). Salmonella causes a number of different disease syndromes ranging from asymptomatic colonisation to severe extra-intestinal illness such as bacteraemia (Butaye *et al*., 2006; Lan *et al*., 2007) meningitis or osteomyelitis (Butaye *et al*., 2006).

The symptoms of salmonellosis include diarrhoea (100% of patients) (Beaudin *et al*., 2002; Leon-Velarde *et al*., 2004; Oh *et al*., 2004; Ehrbar & Hardt, 2005; Biedenbach *et al*., 2006; Butaye *et al*., 2006; Steingroewer *et al*., 2007), fever (80% of patients) (Jones *et al*., 1993; Beaudin *et al*., 2002; Yeh *et al*., 2002; Leon-Velarde *et al*., 2004; Oh *et al*., 2004; Butaye *et al*., 2006; Steingroewer *et al*., 2007), abdominal pain (65% of patients) (Beaudin *et al*., 2002; Leon-Velarde *et al*., 2004; Oh *et al*., 2004; Ehrbar & Hardt, 2005; Butaye *et al*., 2006; Steingroewer *et al*., 2007), vomiting (45% of patients) (Beaudin *et al*., 2002; Leon-Velarde *et al*., 2004; Ehrbar & Hardt, 2005; Butaye *et al*., 2006), blood in the stool (27% of patients) (Beaudin *et al*., 2002; Leon-Velarde *et al*., 2004; Butaye *et al*., 2006), enterocolitis, intestinal inflammation (Ehrbar & Hardt, 2005), headache, nausea (Leon-Velarde *et al*., 2004), septicaemia (Yeh *et al*., 2002) and gastroenteritis (Jones *et al*., 1993; Yeh *et al*., 2002; Olsen *et al*., 2004). According to Olsen *et al. (*2004), a small number of serotypes are associated with systemic, typhoid-like disease.

Lan *et al.* (2007) reported that ST was the most common serovar to cause cardiovascular infections, as well as bone and joint infections. Due to the self-limiting nature of salmonellosis

(Jones *et al*., 1993; Jongerius-Gortemaker *et al*., 2002; Butaye *et al*., 2006; Oh *et al*., 2004) antimicrobial therapy is not usually required. However, Salmonellae are capable of causing severe invasive infections that can have high mortality rates (Biedenbach *et al*., 2006) particularly in immuno-compromised patients such as the very young, the elderly (Biedenbach *et al*., 2006; Steingroewer *et al*., 2007) and AIDS (Acquired Immune Deficiency Syndrome) patients (Lan *et al*., 2007) making antimicrobial therapy essential (Butaye *et al*., 2006).

1.7 Growth requirements

Salmonellae are able to survive and multiply readily in the environment. PT organisms have simple growth requirements and are able to multiply in a wide variety of media (Calnek *et al*., 1991). Acidity, pH, and heat treatment are important factors influencing the growth and survival of pathogens in foods (Jung & Beuchat, 2000). According to Calnek *et al.* (1991), the optimum growth temperature for Salmonella is 37ºC, with the Salmonella organism being susceptible to damage upon exposure to heating (Calnek *et al*., 1991; Jung & Beuchat, 2000; Kang & Fung, 2000) and most common disinfectants (Calnek *et al*., 1991). Salmonellae have an optimum pH of 6.5-7.5 for growth, but can occur at pH of 4.5-9.5 (Jung & Beuchat, 2000).

1.8 Sources of contamination

1.8.1 Eggshell contamination and penetration: Salmonella can be spread by faecal contamination of eggshells during laying or from contaminated nests, floors or incubators after laying (Calnek *et al*., 1991; Turner *et al*., 1998). *Salmonella typhimurium* found in faecal material on the surface of eggs can penetrate the shell and multiply within the egg, allowing PT organisms to be introduced into the incubator with subsequent spread to hatched chicks or poults (Calnek *et al*., 1991). According to Cason *et al. (*1994) infection in the majority of chicks occurs in the incubator after hatching rather than through the eggshell. Salmonella is able to multiply rapidly in the yolk resulting in the infection of the developing embryo. If this embryo hatches, it will serve as a source of infection for the rest of the chicks (Calnek *et al*., 1991).

1.8.2 Direct and vertical transmission: According to Calnek *et al. (*1991), direct ovarian transmission is uncommon in chickens, while Amy *et al. (*2004) reported that vertical transmission can occur by transovarian transmission into eggs. This is supported by Carramiñana *et al*. (2004) who states that Salmonella can be present in the contents of intact egg shells as a result of infection in the reproductive tissue. Chriél *et al.* (1999) reported that a strong association between the ST status of a broiler flock and the parent stock will indicate direct vertical transmission of ST, indicating that the focus for eliminating ST should be at the top of the production pyramid.

1.8.3 Incubator, hatchery, brooder and environment: Contaminated eggshells (Calnek *et al*., 1991), chick fluff and down, dust (Lee *et al*., 1983; Calnek *et al*., 1991) and other hatch

debris may serve as a source of infection in the incubator. According to Calnek *et al. (*1991), Salmonellae can survive in hatchery fluff samples stored at room temperature for up to 5 years.

From the incubator, organisms may be distributed throughout the hatchery by air currents (Calnek *et al*., 1991; Amy *et al*., 2004). According to Cason *et al. (*1994), infection of the chick with Salmonella is likely to occur via the respiratory and digestive systems. Contaminated drinking water could serve as a source of the disease in an infected environment (Lee *et al*., 1983; Calnek *et al*., 1991; Turner *et al*., 1998). PT infection in the brooder is rapidly transmitted by inhalation, faecal contamination of feed and water, or the by the direct consumption of faecal material by the chicks. The disease may also be transmitted by footwear, feedbags, shipping crates or brooding equipment (Calnek *et al*., 1991).

1.8.4 Poultry feeds: Poultry feeds may be a frequent and very important source of PT organisms (Lee *et al*., 1983; Calnek *et al*., 1991; Schiemann & Montgomery, 1991; Ha *et al*., 1998a; Turner *et al*., 1998; Kwon & Ricke, 1999), with meat and bone meal or animal by-products having the highest occurrence of Salmonella organisms. However, Salmonella is not limited to only animal by-product sources, as it has also been detected in plant protein sources including soybean oil meal (Ha *et al*., 1998b). The level of contamination in poultry feeds is usually low, although infection can result from 1 organism/1-15g of feed. *Salmonella typhimurium* can survive for up to 18 months at 11ºC in feed, 16 months at 25ºC, and about 40 days at 38ºC (Calnek *et al*., 1991).

1.8.5 Poultry litter and faeces: According to Calnek *et al. (*1991), ST can survive for at least 18 months at 11ºC in litter; 18 months at 25ºC, and only 13 days at 38ºC. Salmonellae will live for up to 28 months in naturally infected avian faeces. Infestation of Salmonella will be maintained by the cycling of the organism between the litter and the intestinal tract, although litter build-up from continuous use without changing will be inhibitory to Salmonellae (Ricke *et al*., 1997). This is supported by Rigby & Pettit (1979) who reported that as infected flocks on litter mature, fewer birds shed the organism, the number of infected birds' decreases, and the litter itself becomes inhibitory to Salmonella.

1.9 Routes of infection: It is important to find out exactly how infection can be introduced into a Salmonella-free breeding flock (Jordan & Pattison, 1996). Figure 2.2 shows possible routes of infection.

1.10 Pathogenicity

1.10.1 Young chickens: According to Hinton *et al.* (1990), young chickens are more susceptible to Salmonella colonisation than older birds. The number of ST bacteria required for colonisation (by oral gavage) in a chicken at one day of age is roughly 250 bacteria, with

only 105 bacteria being required at three days of age (Chriél *et al*., 1999). This is in disagreement with Calnek *et al*. (1991) who states that the older the chicken, the greater the number of ST organisms required to infect them (Calnek *et al*., 1991).

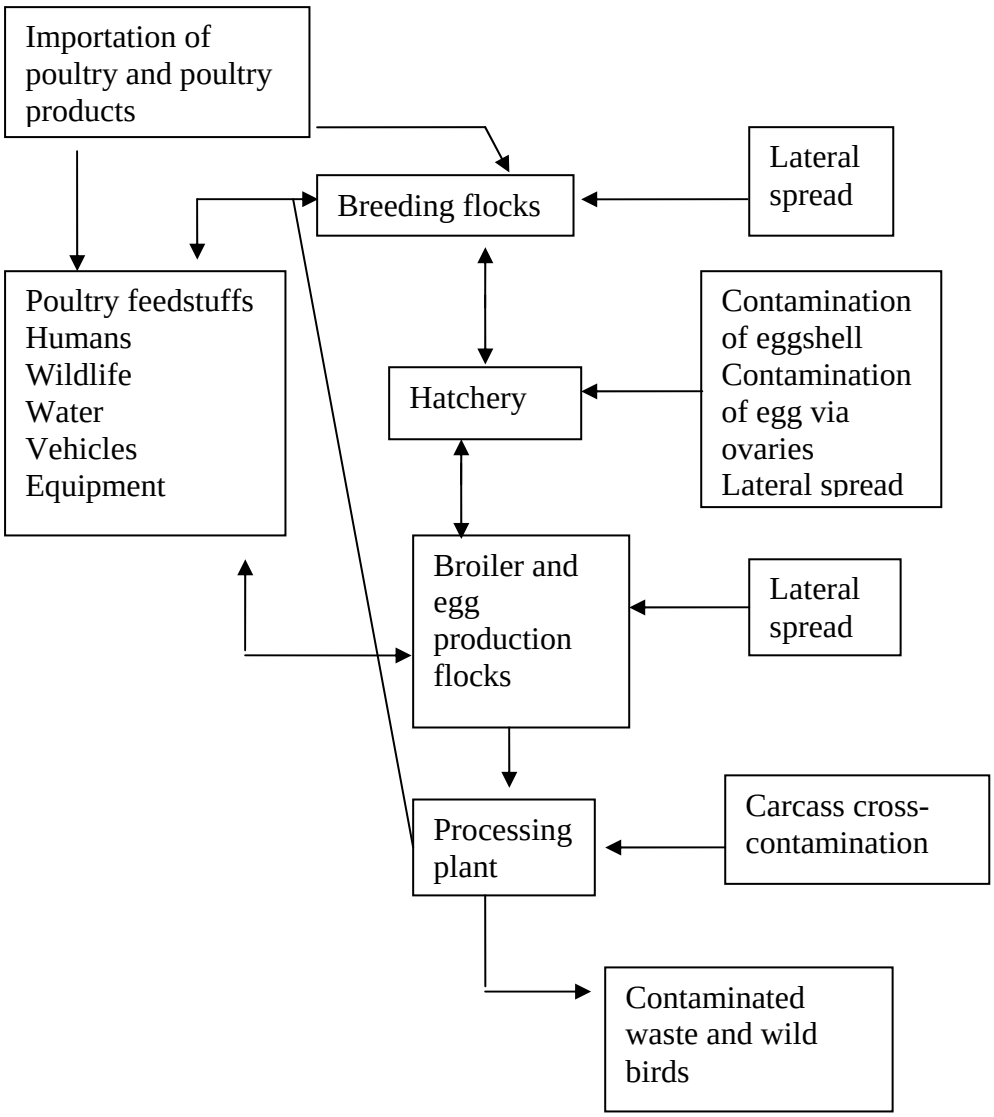

Figure 2.2 Cycle of Salmonella infection (Jordan & Pattison, 1996)

Mortality rates for chicks reared under natural conditions can vary from insignificant to 10-20%, although mortality rates of 80% or higher can be encountered in severe outbreaks. Mortality rates will vary depending on the environment, strain of infecting organism, and presence of associated infections (Calnek *et al*., 1991). According to Beal *et al.* (2004, 2006), the age at primary infection with ST will have a noticeable effect on the persistence of infection as well as a smaller effect on immunity to a subsequent re-challenge. According to Beal *et al.* (2006) chickens over 3 weeks of age produce a strong humoral and cellular

immune response following infection with ST, and infection will rarely cause clinical disease in chicks over 3 weeks of age (Calnek *et al*., 1991; Beal *et al*., 2006).

1.10.2 Adult birds: Adult birds infected with PT organisms will generally exhibit no external signs of infection, and they may serve as asymptomatic intestinal carriers of the organism (Calnek *et al*., 1991; Beal *et al*., 2004). PT infections are not selective in their pathogenicity for specific strains or breeds of birds (Leaney *et al*., 1978; Calnek *et al*., 1991).

1.10.3 Turkeys: PT infections are more common in turkeys than in any other domesticated avian species, with *Salmonella typhimurium* responsible for approximately 50% of the PT outbreaks (Calnek *et al.,* 1991).

1.10.4 Geese, ducks, pigeons: Young geese and ducks are relatively susceptible to PT infection. Pigeons surviving PT outbreaks will frequently become chronic carriers, excreting the organisms sporadically in their faeces (Calnek *et al*., 1991).

1.10.6 Other animals: PT's are common pathogens of all species of domestic and wild mammals. Cattle (Veling *et al.,* 2002), swine, sheep, goats, dogs, cats, horses, mink, foxes, and reptiles are among the many animal species that may be asymptomatic carriers of the organism. Rats and mice are frequently intestinal carriers of PT organisms, particularly ST and SE (Calnek *et al*., 1991; Okamura *et al.,* 2007).

1.11 Mode of action

1.11.1 In poultry: *Salmonella typhimurium* causes infection by attaching to the mucosal surfaces of the intestine and then invading and penetrating into the epithelial cells (Berndt & Methner, 2004). Upon infection by Salmonellae, enterocytes indicate infection to the immune system. Salmonella flagellin protein is identified by Toll-like receptor 5 (TLR-5) in host cells leading to the release of inflammatory cytokines and chemokines by various host cells. This release leads to the attraction and activation of polymorphonuclear leukocytes resulting in acute inflammation (Vo *et al*., 2007).

Salmonella spp. rely on a wide range of virulence factors to colonise, replicate and spread within a host, as well as to neutralise host defences (Calnek *et al.,* 1991; Amy *et al*., 2004; Ehrbar & Hardt, 2005). Salmonella virulence genes are either scattered in the chromosome or clustered within Salmonella Pathogenicity Islands (SPI) (*Reis et al., 2003;* Ehrbar & Hardt, 2005*).* The SPI-1-encoded Two type III secretion systems (TTSS) are gut associated and allow the invasion of the epithelium and induce inflammatory signals (Olsen *et* al., 2004; Ehrbar & Hardt, 2005). TTSS are widely distributed among Gram-negative animal and plant pathogens. They are responsible for the injection of bacterial proteins into eukaryotic host cells (Calnek *et al.,* 1991; Amy *et al*., 2004; Ehrbar & Hardt, 2005).

Some Salmonella strains are able to mutate frequently bringing about permanent changes in their tolerance, resistance and or virulence. This poses a problem for scientists wishing to study these bacteria as such Salmonella populations are in a state of frequent change (Humphrey, 2001).

1.12 Clinical signs

Non-host specific Salmonella will generally not cause disease in poultry (Tan *et al*., 1997a), and is usually seen in chicks, poults or ducklings younger than 2 weeks of age and is very rarely found in birds over 4 weeks of age. The morbidity and mortality seen in poultry varies considerably (Calnek *et al*., 1991; Jordan & Pattison, 1996). The clinical signs are not specific and will be similar regardless of the serotype of Salmonella involved (Jordan & Pattison, 1996).

1.12.1 Young birds: Signs of PT infections in all species of young poultry are very similar. Birds will stand dejectedly in one position with lowered heads, closed eyes, drooping wings, and ruffled feathers. Some birds may show signs of anorexia. Increased water consumption may occur, causing prolific, watery diarrhoea resulting in pasting of the vent. Infected birds will show a tendency to cluster together near heat sources. Blindness and conjunctivitis in chicks is also frequently associated with salmonellosis (Calnek *et al*., 1991; Jordan & Pattison, 1996).

1.12.2 Adult birds: Mature poultry will generally exhibit no outward signs of infection (Calnek *et al*., 1991; Jordan & Pattison, 1996; Tan *et al*., 1997a). Signs of acute infection will include decreased appetite, increased water consumption, diarrhoea, dehydration, and general apathy. In most cases recovery is rapid, and losses due to death will usually not exceed more than 10% (Calnek *et al*., 1991; Jordan & Pattison, 1996).

1.13 Post-mortem findings

1.13.1 Young birds: Lesions may be entirely absent in extremely severe outbreaks. The symptoms most commonly observed in advanced cases are emaciation, dehydration, coagulated yolks, congested liver and spleen with hemorrhagic streaks or pinpoint necrotic foci, congested kidneys, and pericarditis (Calnek *et al*., 1991; Jordan & Pattison, 1996).

1.13.2 Adult birds: Congestion and swelling may be seen in the liver, spleen, and kidneys of acutely infected birds, with signs of hemorrhagic or necrotic enteritidis, pericarditis, and peritonitis (Calnek *et al*., 1991). The most distinctive post-mortem finding, seen in approximately one-third of mortalities, is typhlitis, with the caeca distended by hard white necrotic cores (Jordan & Pattison, 1996). Necrotic ulcers in the intestines; nodules on the heart; and distorted ovules may occasionally be found (Calnek *et al*., 1991 Jordan & Pattison, 1996).

1.14 Diagnosis

According to Calnek *et al.* (1991), clinical observations and necropsy findings can be used in conjunction with flock history to determine whether a PT infection has occurred. However, confirmation of the diagnosis will require the isolation and identification of the causal agent (Calnek *et al*., 1991; Jordan & Pattison, 1996).

Several methods have been developed to collect samples from the environment as an indirect indication of flock infection. These methods include samples from nest or floor litter, dust and drag swabs (Jordan & Pattison, 1996).

PT organisms may only be sporadically shed in the faeces thereby limiting the reliability of cloacal swab cultures as a diagnostic procedure; therefore, failure to isolate the organisms will not necessarily prove the absence of infection. Fluff samples collected on hatch days are a very effective method for the early detection of PT infections. The bacteriologic examination of yolk material from embryos that died between days 19 and 21 is a practical method for the detection of carrier flocks (Calnek *et al*., 1991).

1.15 Prevention and control

1.15.1 Breeder flocks: In breeder flocks routine bacteriologic monitoring of the grandparent flocks and their progeny can help to produce parent stock that is free of Salmonellae (Calnek *et al*., 1991). Flocks that have previously carried the infection should never be used as a source of hatching eggs, even if they have been on antibiotic therapy (Calnek *et al*., 1991; Chriél *et al*., 1999). An active rodent eradication campaign is a crucial part of any Salmonella control program. Dogs, cats, sheep, cattle, horses, swine, and wild birds should not have access to any poultry production sites (Calnek *et al*., 1991).

1.15.2 Hatchery and egg sanitation: The introduction of infection into the incubator via faecal material will be decreased by using only clean eggs for hatching purposes. Egg-dipping and –spraying procedures can be utilised to destroy any Salmonellae present on the surface of hatching eggs. Incubators should be thoroughly cleaned of all hatch debris and then washed, disinfected, and fumigated after hatching. Strict bio-security measures should be in place (Calnek *et al*., 1991). Hatcheries should attempt to avoid pooling the eggs from different parent flocks in the incubators in order to minimise the risk of spreading Salmonella from infected flocks to chickens from non-infected parent flocks (Chriél *et al*., 1999).

1.15.3 Feed: Heat treatment, chemical disinfection and irradiation are just some of the methods that have been proposed for eliminating Salmonella spp. in feeds. Unfortunately the high costs involved, decline in nutrient quality, corrosiveness, worker safety and the potential for recontamination have prohibited the application of most of these methods (Ha *et al*., 1998a, 1998b).

1.15.4 Vaccines: There are a number of both live and killed Salmonella vaccines used commercially worldwide. Live attenuated vaccines are generally more effective in controlling salmonellosis. However, the potential for reversal to virulence through horizontal gene transfer remains a concern for live attenuated vaccines (Okamura *et al*., 2007). According to Tan *et* al. (1997a; 1997b) some researchers reported that vaccination was protective, while others indicated that it was not effective.

1.15.5 Chemotherapy: Chemotherapy can be defined as the use of sulphonamides, antibiotics and nitrofurans for the treatment of PT infections (Calnek *et al*., 1991). According to Barrow *et al.* (1988), both chemotherapeutic and growth-promoting antibiotics have been shown to have an influence on Salmonella excretion. Chemotherapeutic antibiotics will directly inhibit Salmonella, while growth promoters will act indirectly by altering the micro-ecology of the gastrointestinal tract.

2. Antibiotic growth promoters (AGPs)

2.1 History

Antimicrobials were introduced into human chemotherapy in the 1940s, and soon after they were introduced into veterinary practice (Witte, 2000), finding widespread use in the livestock industry as therapeutic agents and growth promotants (Joerger, 2003; Skjolaas *et al.,* 2007).

The UK “Swann Report” of 1969 recommended that there should be antimicrobials dedicated to human medicine only and that the use of overlapping AGPs used in both human medicine and animal production should be discontinued (Humphrey, 2001). These recommendations led to changes in the European Unions’ feed additives regulation in the early seventies (Wegener, 2003).

By the middle of 1999, the use of bacitracin, spiramycin, tylosin and virginiamycin as growth promoters was banned throughout the EU on the recommendation of the "precautionary principle". These AGPs belonged to classes of antimicrobials also used by humans and were considered to have “unacceptable occupational toxicity risks” (Dibner & Richards, 2005; Phillips, 2007). These antibiotics were banned even though evidence of the actual risk to human health was insufficient (Phillips, 2007).

Few attempts have been made to either prove or disprove the professed risk to human health, resulting in conflicting views being held in this regard. The possibility that the use of AGPs in food animals might benefit human health seems to be considered irrelevant by those that support the banning of antibiotics (Phillips, 2007).

2.2 What is an AGP?

Antimicrobial substances are drugs that are indispensable in the treatment of bacterial

infections in humans. In the livestock industry, antimicrobials are used as growth promoters, for prophylaxis and for the treatment of bacterial infections (Frei *et al*., 2001; Phillips, 2007).

2.3 Zinc Bacitracin

Bacitracin (BC) is one of the most common antibiotics used worldwide as an animal feed additive largely due to its growth-promoting effects (Capitan-Vallvey *et al*., 2002; Engberg *et al*., 2000). It is a basic and cyclic polypeptide antibiotic produced by the strains of *Bacillus licheniformis* and *Bacillus subtilis* (Capitan-Vallvey *et al*., 2002; Van Poucke *et al*., 2003).

Commercial BC is frequently used in association with zinc to improve growth rates and feed conversion in poultry, pigs and cattle. In 1999, the European Union banned its use as an animal feed additive (Capitan-Vallvey *et al*., 2002; Van Poucke *et al*., 2003).

AGPs, including zinc bacitracin (Zn-BC), are generally not absorbed from the intestine at the dietary concentration used in commercial rations. Zn-BC acts within the gastrointestinal tract to modify the intestinal microflora and gut wall structure. The performance-enhancing effect of Zn-BC has been observed in different species of poultry, including turkeys, broiler breeders, laying hens, and broiler chickens (Huyghebaert & de Groote, 1997).

2.4 Mode of action

Antibiotic use in livestock has been linked to various physiological, nutritional and metabolic effects on growth. Some of these effects include nutrient protection from microbial breakdown thereby increasing the nutrient availability to the host (Huyghebaert & de Groote, 1997; Skjolaas *et al*., 2007); thinning of the gastrointestinal barrier to allow for better nutrient absorption; decreased microbial toxins, and reduced sub-clinical intestinal infections (Skjolaas *et al*., 2007).

Early trials conducted on germ-free animals showed that oral antibiotics did not have growth-promoting effects. Subsequent studies on the mechanisms for growth promotion have focused on the interaction between the antibiotic and the gut microbiota. AGPs are able to act directly on the gut microflora in order to decrease the competition for nutrients. AGPs will also reduce microbial metabolites that are capable of depressing growth (Huyghebaert & de Groote, 1997; Dibner & Richards, 2005; Gunal *et al.,* 2006). AGPs are able to enhance nutrient digestibility in germ-free animals by reducing gut size (Dibner & Richards, 2005).

2.5 Effects on poultry production

Dahiya *et al. (*2006) found that the net effect of using antibiotic growth promoters in the poultry industry was estimated to be a 3–5% increase in growth and feed conversion efficiency. Knarreborg *et al. (*2004) reported that the ileal absorption coefficients of total and individual fatty acids were generally greater in birds fed the antibiotic-supplemented diets

compared with those fed the un-supplemented diets, while Collignon (2004) states AGPs show little efficacy in improving overall animal health or welfare.

2.6 Effect on other animal production

The advantages of low-dose antimicrobials in diets for weanling pigs are numerous and include improvement in average daily weight gain and feed efficiency (Kieke *et al*., 2006; Skjolaas *et al*., 2007).

2.7 Antibiotic resistance

It has been argued that the continuous, unregulated and excessive use of AGPs in livestock feeds will impose a selection pressure for antibiotic resistant bacteria (Dahiya *et al*., 2006), generating a reservoir for these bacteria (Beaudin *et al*., 2002; Antunes *et al*., 2003; Carramiñana *et al*., 2004; De Oliveira *et al*., 2005; Berchieri *et al*., 2006).

Most antimicrobial-resistant Salmonella infections will occur from eating contaminated foods of animal origin. Antibiotic resistance in Salmonella will limit the therapeutic options available to veterinarians and physicians in the treatment of certain human cases of salmonellosis. (Beaudin *et al*., 2002; Antunes *et al*., 2003; Carramiñana *et al*., 2004; De Oliveira *et al*., 2005; Berchieri *et al*., 2006).

2.7.1 Acquisition of resistance genes: Of the more than 150 antibiotics that have been developed by modern medicine, bacteria have evolved the means to interfere with many of the drugs' actions through the use of bacterial resistance genes which code for beta-lactamases, transferases, and other enzymes. The enzymes may break down the drug or alter its target in the bacterial cell. The bacteria may also expel the antibiotic through an efflux system (Michael *et al*., 2006; Mlot, 2000).

2.8 AGP replacement products

2.8.1 Vaccination: According to Van den Bogaard & Stobberingh (2000), AGP use can be minimised by the optimal usage of existing vaccines and the development of new vaccines. The vaccination of birds will result in an increased resistance to Salmonella (Berndt & Methner, 2001).

2.8.2 Mycotoxins: Many of the mycotoxins that have the ability to contaminate poultry feed will have antimicrobial properties. Mycotoxins may destabilise the gut flora and therefore affect the feed efficiency of the bird even before toxicity signs appear (Collignon, 2004).

2.8.3 Bacteriocins: Bacteriocins are produced by bacteria and are lethal to any other bacteria other than the producing strain. Bacteriocins may be used to achieve a competitive

advantage, and the bacteria which produce these compounds may therefore have a role to play as part of competitive exclusion preparations (Joerger, 2003).

2.8.4 Bacteriophages: According to Dahiya *et al. (*2006), bacteriophages are viruses that are able to infect bacterial cells, replicate within these cells, destroy the bacterial host by lysis and then release new bacteriophages into the host. Bacteriophages may have a role to play as an alternative to antibiotic control of bacterial replication.

2.8.5 Antimicrobial peptides: Peptides can influence intestinal microbiota in the same way as antibiotics. They can be used to inhibit microbial growth on surfaces as well as in biological material such as vaccines (Joerger, 2003).

2.8.6 Cytokines/ Ig: After infection by pathogens or after vaccination, cytokines are responsible for determining the type and extent of immune response that will follow. Cytokines could be used as naturally occurring therapeutics and vaccine adjuvants (Lowenthal *et al*., 2000).

2.8.7 Chicken interferon gamma: Chicken interferon gamma is an immune cell product that has showed promise as a growth promoter and can be used to treat infections (Mlot, 2000).

2.8.8 Natural alternatives: Herbs can enhance antimicrobial activity as well as stimulate the endocrine and immune systems. Apart from the enhanced welfare benefit of using natural products, they can help to stimulate the metabolic and immune status within the animal. Various natural ingredients can have beneficial effects on the digestive system of animals as well as on the gut microflora. *Origanum vulgare*, *Piper nigrum*, *Syzygium aromaticum* and *Thymus vulgaris*, thymol, carvacrol, curcumin, piperin and eugenol have been reported to have antibacterial effects against Clostridia and other bacteria such as *E. coli*, *S. aureus*, *Salmonella typhimurium*, *Listeria monocytogenes* and *Y. enterocolotica* (Dibner & Richards, 2005; Dahiya *et al*., 2006; Lee *et al*., 2006; Skjolaas *et al*., 2007).

2.8.9 Organic acids: Organic acid-based products can be designed to inhibit the growth of undesirable microorganisms in both raw materials and finished feed products. The mode of action of organic acids against microorganisms is still not fully understood (Hume *et al*., 1993; Ha *et al*., 1998; Hinton *et al*., 1999; Jung & Beuchat, 2000; Dibner & Richards, 2005; Berchieri *et al*., 2006; Dahiya *et al*., 2006).

2.8.10 Prebiotics: Prebiotics can benefit the host by selectively stimulating the growth and activity of bacteria in the colon. Prebiotics can increase fermentation both *in vitro* and *in vivo.* There appears to be a synergistic effect between prebiotics and the indigenous microflora

found in the gut that has the potential to protect poultry against *Salmonella* spp. (Dibner & Richards, 2005; Dahiya *et al*., 2006; Donalson *et al*., 2007).

2.8.11 Probiotics: Probiotics have the potential to improve gut health and feed efficiency, by creating an environment that accelerates the establishment of a beneficial and stable gut flora. The emphasis shifts from working against, to working with, the natural ecology of the gut (Joerger, 2003; Dibner & Richards, 2005; Gunal *et al*., 2006; Skjolaas *et al*., 2007).

2.8.12 Competitive exclusion (CE): Competitive exclusion occurs when one population of microorganisms is unable to colonise the gut because of the presence of another population of microorganisms which prevent their attachment to the intestinal epithelium (Bolder *et al*., 1992). Dosing of day-old chicks with flora from healthy adult birds can enhance pathogen resistance (Hinton *et al*., 1990; Calnek *et al*., 1991; Nisbet *et al*., 1996; Hoszowski & Truszczyński, 1997; Joerger, 2003).

3. Immunity

3.1 Cellular basis of the response

Cell-mediated immune response plays an important role in host protection in chickens. Salmonellosis produces a local cellular immune response that is regulated by various inflammatory mediators. This response leads to infiltration of the GIT by immune effector T lymphocytes, heterophils and macrophages. Increased levels of circulating antibodies are detected during the early phase of infection indicating that humoral immune response may play an important role in protective immunity (Sasai *et al*., 1997; Tizzard, 2002; Bar-Shira *et al*., 2003; Bar-Shira & Friedman, 2006).

B cells and plasma cells are responsible for the production and secretion of antibodies. Avian B cells are mainly located in the major lymphoid organs, the spleen in particular. Avian B-cell precursors originate from stem cells in the yolk sac and then in the bone marrow, from where they migrate to the bursa of Fabricius. In the bursa the pre-B cells undergo processing and selection finally ending up to as mature B cells which are then able to migrate to secondary lymphoid organs (Tizzard, 2002; Bar-Shira *et* al., 2003; He *et al*., 2007).

Mucosal surfaces lining the respiratory, digestive and genitourinary tracts are the main entry point for infectious agents, including Salmonella, into the body. The mucosa-associated lymphoid tissues (MALT) have evolved specialised features that aid them in their role as the first line of defence by lowering the ability of pathogens to adhere to the epithelium thereby reducing colonisation (Fukutome *et al*., 2001; Asheg *et al*., 2002; He *et al*., 2007). Continual desquamation and renewal of the gut epithelium may play a key defensive role in the intestine (Sakata, 1987; Bar-Shira *et al*., 2003; Montagne *et al*., 2003).

A major component of MALT is the gut-associated lymphoid tissue (GALT). GALT is responsible for inducing immune responses against bacterial, viral and parasitic antigens that may be introduced via the digestive system. The GALT consists of amongst others, the bursa of Fabricius, and caecal tonsils (CT), Peyer's patches (PP) and intraepithelial lymphocytes (IEL) (Bar-Shira *et al*., 2003).

Pathogenic bacteria are able to penetrate the GIT proceeding into deeper tissues, the lymphatic system and eventually into the blood, resulting in systemic infections. ST and SE produce a more localised infection in the intestinal epithelium, resulting in damage and loss of functionality of the intestinal mucosa, causing severe diarrhoea since this barrier is essential for maintaining ionic homeostasis (Sakata, 1987; Bar-Shira *et al*., 2003; Montagne *et al*., 2003).

Minor disturbance and dysfunction of the intestinal epithelium may lead to diarrhoea, constipation, malnutrition, dehydration, infectious disease or intestinal inflammatory diseases, all of which clearly indicates just how essential the correct functioning of this barrier is to animal health (Zigterman *et al*., 1993; Hooper *et al*., 1998).

3.2 The bursa of Fabricius

The bursa of Fabricius is an organ unique to birds (Tizzard, 2002; He *et al*., 2007). It is a hollow sac located just above the cloaca. The bursa will reach its maximum size a few weeks after hatching and will then begin to gradually decrease in volume. The bursa contains folds of epithelium with thousands of lymphoid follicles extending into the lumen. These bursal follicles consist of more than 90% B cells (Tizzard, 2002).

3.3 Other lymphoid tissues

B cells migrate from the bursa of Fabricius to the secondary lymphoid organs, which include amongst others, the spleen, the GALT including the CT and PP, Harderian glands and the paranasal glands. It is in these organs that the B cells will encounter antigens and begin to synthesise and secrete antibodies (Tizzard, 2002; Bar-Shira *et al*., 2003).

3.4 Immunoglobulin classes

Immunoglobulins are glycoproteins of which 3 classes are found in birds, namely IgM, IgY, and IgA. These immunoglobulins are distributed in body fluids and aid in the defence of the body against pathogens. Immunoglobulin Y (IgY) is found in blood and egg yolk serum. Immunoglobulin A (IgA) occurs in egg white, intestinal secretions, and bile. IgA is the predominant class of immunoglobulin and serves as one of the many forms of frontline defence against pathogens such as Salmonella (Schiemann & Montgomery, 1991; Tizzard, 2002). The oviduct is able to produce IgA which it secretes with albumin as the fertilised ovum

moves down the oviduct. Immunoglobulin M (IgM) can be detected in serum and egg white, seminal plasma, in bile, as well as in intestinal contents (Tizzard, 2002).

3.5 The antibody response

IgM is the immunoglobulin predominantly found during primary immune responses after the initial exposure to an antigen. This response is short lived and is followed by production of IgY antibodies. IgA production in birds occurs on body surfaces, especially mucus membranes, and generally, live organisms are required to obtain a good IgA response (Schiemann & Montgomery, 1991; Tizzard, 2002).

3.6 Yolk antibodies and passive immunity

According to Bar-Shira & Friedman (2006), immune protection is provided during the first week of life by maternal antibodies and innate effector mechanisms. Maternal antibodies provide passive protection against pathogens which protect chicks for around two weeks until their own immune system fully matures (Klipper *et al*., 2004). Maternal antibodies enhance the antibody response to initial challenge by pathogens, thereby influencing the developing immune system of the chick (Abdel-Moneim & Abdel-Gawad, 2006). One of the drawbacks to maternal antibodies is that they hinder vaccination by blocking vaccine antigens before they are able to produce an effective immune response (Klipper *et al*., 2004).

4. Cyclophosphamide

Cyclophosphamide (CY) is a non-specific immunosuppressant agent affecting primarily antibody-mediated immunity (Ettinger & Hirata, 1982; Hemendinger & Bloom, 1996; He *et al*., 2007). Immunosuppressed flocks are susceptible to increased incidences of secondary infections; they will have poor feed conversion ratios, as well as a reduced protection response to most commonly used commercial vaccines (He *et al*., 2007). Cyclophosphamide is widely used in organ transplantation and the treatment of various autoimmune disorders (El-Abasy *et al*., 2004), and is able to suppresses avian immune responses. Cyclophosphamide causes depletion of B-lymphocytes and suppresses humoral immunity (Corrier *et al*., 1991; He *et al.,* 2007) without impairing thymic functions (Lam & Hao, 1987).

The ability of CY to act as an immunosuppressive agent has been investigated in a variety of laboratory mammals, as well as in humans and chickens. The consensus that was reached indicated that CY could act as a potent immunosuppressive agent to humoral antibody production, but that the effect could fluctuate considerably according to species, dosage, time of administration and method of evaluation (Ettinger & Hirata, 1982).

It was reported by El-Abasy *et al*. (2004) that the injection of CY into newly hatched chicks was able to induced B lymphocyte cell damage and result in irreversible humoral immunosuppression. Animals that already had poor humoral immunity showed increased

susceptibility to infection. Desmidt *et al.* (1998) found that, in chickens, CY was able to induce a complete and long-term immunosuppression of the antibody response (Desmidt *et al.,* 1998).

Ettinger & Hirata (1982) found that the administration of CY during the first 4 days post-hatch would suppress natural agglutinin titres for prolonged time periods, namely 6 to 12 weeks. This did not hold true for chicks that were administered with CY 7 days post-hatch where it was difficult to suppress the natural antibody levels for prolonged time periods. It would also appear that the longer the treatment with CY, the greater the suppression of natural agglutinin titres.

5. Gut microflora

There are three major components to GIT health, with each component, namely the diet, the mucosa, and the commensal flora interacting and working with each other to maintain a dynamic equilibrium (Fig. 2.3). A delicate balance is needed to ensure this maintenance of gut health. Numerous obstacles stand in the way of this delicate equilibrium including potentially pathogenic enteric bacteria capable of disturbing digestive function, negatively affecting growth rates and even capable of causing death. The diet of the bird will have an important influence on gut health, proving to be either beneficial or harmful (Montagne *et al*., 2003; Dibner & Richards, 2005). There are numerous bacterial populations present in the GIT, with as many as 500 bacterial species being present in the GIT microflora of chickens. These populations are capable of exerting profound effects on the overall health, development and performance of the bird (Dibner & Richards, 2005; Dahiya *et al*., 2006).

The Nurmi principle (in poultry science) proposes that “one can improve the survival of chicks by inoculating them at an early age with adult gut microbiota” (Bar-Shira *et al*., 2003; Montagne *et al*., 2003; Dibner & Richards, 2005). After 2 to 3 weeks, the intestinal microflora will be established and stable (Dibner & Richards, 2005).

The benefits that the bird may receive from the presence of these bacteria can come at a cost as the bacteria may compete with the host for nutrients, they may secrete compounds that are toxic to the bird and may cause an immune and/or inflammatory response in the GI tract (Dibner & Richards, 2005). There is an advantage to the presence of indigenous intestinal microflora, as they are able to act as a defence mechanism against enteric pathogens by preventing their establishment in the GIT of the bird, this is also known as “competitive exclusion” or “colonisation resistance”. This protective mechanism can be disrupted when the normal equilibrium is disturbed by the use of antimicrobial agents (Bar-Shira *et al*., 2003; Montagne *et al*., 2003; Dibner & Richards, 2005; Dahiya *et al*., 2006).

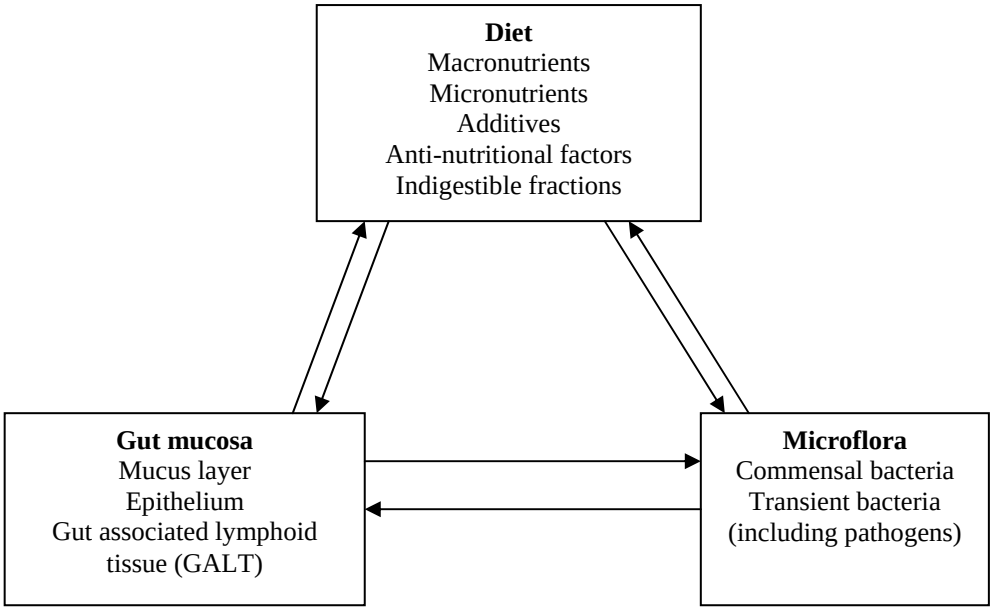

Fig. 2.3 Schematic representation of the gut ecosystem (Montagne *et al*., 2003)

Bacterial antagonism is an important factor in colonisation resistance. Bacterial antagonism is "the inhibition of growth or reduction in number of one bacterial species by one or more other bacterial species". Indirect bacterial antagonism can alter the composition of the GIT microbial biota through altering physiologic responses or products produced by the host animal. The removal of some bacterial strains from the GIT microbiota, such as through the use of antimicrobial agents, will increase the concentrations of the limiting nutrients that supported these strains. These nutrients would then be available to support the growth of other bacteria, including enteric pathogens (Bar-Shira *et al*., 2003; Montagne *et al*., 2003; Dibner & Richards, 2005; Dahiya *et al*., 2006).

Normal microflora will encourage the development of the mucus layer; the epithelial monolayer; and the lamina propria, thereby stimulating the development of the hosts' intestinal defences. The mucus layer prevents pathogenic microbes from attaching and entering into the hosts' tissues. If the mucus layer is penetrated by the microbes the epithelium will then act as the next barrier, followed by the lamina propria with its system of immune cells to provide antibodies, cytotoxic and helper T cells, and phagocytic cells to defend the host against the pathogen invasion (Bar-Shira *et al*., 2003; Montagne *et al*., 2003; Dibner & Richards, 2005).

The development of the mucus layer can also have a negative impact on the host, as several bacterial species are able to enzymatically digest this layer causing the host to continually secrete more mucus. This can be seen as an inefficient utilisation of the bodies' energy store,

as the high cell turnover, increased rate of metabolism and protein synthesis are responsible for up to 23 to 36% of the energy expenditure by the body (Dibner & Richards, 2005).

Antibiotics, organic acids, probiotics, prebiotics, trace minerals, enzymes, herbs and spices are amongst some of the many products currently being sold with the goal of positively altering the GIT microflora (Dahiya *et al.,* 2006; Lee *et al.,* 2006; Donalson *et al.,* 2007; Skjolaas *et al.,* 2007). Where law has not restricted it, the use of antibiotics is the most commonly used dietary intervention to alter the gut microflora (Dibner & Richards, 2005).

Chapter 3

Pilot trial for determining optimum conditions to obtain *Salmonella typhimurium* infection in broiler chicks

Salmonella typhimurium (ST) was used to challenge broilers in this trial as the parent flocks of commercial broiler chicks are vaccinated against *Salmonella enteritidis* (SE), resulting in a carry-over of maternal antibodies against *S. enteritidis* and therefore a natural resistance against this bacterium during early life. Although commercial parent flocks are routinely vaccinated against SE and not ST, it is possible that the chicks may have circulating maternal antibodies against ST. For this reason, the necessity of administering an immunosuppressive agent such as cyclophosphamide was evaluated.

Cyclophosphamide (CY) is an immunosuppressive agent, widely used in organ transplantation and the treatment of various auto-immune disorders (El-Abasy *et al*., 2004), that suppresses avian immune response. Cyclophosphamide causes depletion of B-lymphocytes and suppresses humoral immunity (Corrier *et al*., 1991; He *et al.,* 2007). It has been reported that injection of CY to newly hatched chickens primarily induced selective B lymphocyte cell damage resulting in irreversible humoral immunosuppression. Animals with deficient humoral immunity resulted in increased susceptibility to infection (El-Abasy *et al*., 2004). In chickens, CY induces a complete and long-term immunosuppression of the antibody response against specific antigens (Desmidt *et al.,* 1998).

Sensitivity of natural antibody forming cells to CY depends upon dosage and age (Ettinger & Hirata, 1982). Treatment of chickens during the first few days of life with CY has been shown to suppress humoral antibody production without impairing thymic functions (Lam & Hao., 1987). Administration of CY during the first 4 days post-hatching resulted in suppression of natural agglutinin titres, which lasted throughout the testing period of 6 to 12 weeks. However, when the CY injection was initiated on the 7th day of age, it was difficult to suppress the natural antibody levels for a prolonged period of time (Ettinger & Hirata, 1982).

The aim of this trial was to determine the concentration of *Salmonella typhimurium* that is needed to infect broiler chickens, without causing mortalities or extreme discomfort to the chickens. For the same reasons the ideal age of exposure to ST was evaluated as susceptibility to infections are very high during the first 1-3 days of age and decline thereafter (Calnek *et al*., 1991; Beal *et al.,* 2004). The second aim of this trial was to determine if cyclophosphamide administration is necessary and if it will suppress the humoral immune system sufficiently to allow infection with ST of commercial broiler chicks.

3.1 Materials and methods

3.1.1) *Chickens*: A total of 380 commercial Ross 788 broiler eggs were obtained from Eagle's Pride Hatchery (Pretoria, South Africa). The eggs were set at the hatchery facilities on the Research Farm of the University of Pretoria (Hatfield, Pretoria). A total of 264 first-grade chicks were randomly selected at hatch and placed into 66 pens with 4 chicks per pen. The chicks were not sexed. The chicks were screened for Salmonella by means of a faecal swab sample taken on day 1 of the trial prior to the challenge of the birds with Salmonella culture on either day 4 or 7.

3.1.2) *Experimental design*: The trial was conducted in 3 separate broiler facilities on the Research Farm of the University of Pretoria (Hatfield, Pretoria). One facility housed a Salmonella-free control group which received 2 levels of cyclophosphamide (CY), each with 3 replicates and 4 chicks per replicate. Starting on the day of hatch and continuing for the first four days of the trial the chicks were injected intramuscularly (IM) daily with either 1mL of a saline solution containing 3mg CY/mL or with 1mL of saline solution without CY. These control chicks received 0.2mL of sterile saline solution via oral gavage at day 4.

The remaining two facilities housed Salmonella-exposed chicks with the only difference between these 2 facilities being the day of Salmonella challenge, namely day 4 or day 7 of the trial. There were 10 treatment groups within each of the two facilities comprising of 5 different Salmonella levels, each of these being subdivided into 2 levels of CY (0 and 3mg). Each treatment was replicated 3 times with 4 chicks per replicate. The 5 Salmonella levels were attained by oral gavage of 0.2mL of a Salmonella suspension with a concentration of either 1 x 10^6 CFU/mL, 1 x 10^7 CFU/mL, 1 x 10^8 CFU/mL, 1 x 10^9 CFU/mL or 1 x 10^{10} CFU/mL.

3.1.3) *Bacteria*: A culture of a Naladixic Acid Resistant strain of *Salmonella enterica* subsp. enterica serovar Typhimurium was obtained from the Veterinary Institute Onderstepoort, South Africa. The Salmonella suspension was administered on either day 4 or day 7 of the trial via oral gavage. All chicks received 0.2mL of the Salmonella culture at one of five different concentrations, namely 1 x 10^6 CFU/mL; 1 x 10^7 CFU/mL; 1 x 10^8 CFU/mL; 1 x 10^9 CFU/mL and 1 x 10^{10} CFU/mL. Chicks in the Salmonella-free control group received 0.2mL of saline solution via oral gavage. The methods used in the culturing of the bacteria can be found in the Appendix.

3.1.4) *Cyclophosphamide (CY)*: Cyclophosphamide (CY, Sigma-Merck, Germany) was administered to half of the chicks via intramuscular injection of 3mg CY dissolved in 1mL of saline solution once daily for the first four days of the trial. The chicks that did not receive the CY were injected with 1mL of saline solution once daily for the first four days.

3.1.5) *Husbandry*: The experiments were conducted in environmentally controlled broiler houses fitted with concrete floors and covered with wood shavings as bedding material. Each replicate of the various treatments were kept in individual pens with an area of 1.5m^2 and an open space of approximately 50cm between adjoining pens to prevent direct contact between chicks of different replicates. Each pen was equipped with infra-red heating lamps, tube feeders, bell drinkers and fountain drinkers. Chicks received feed and water on an *ad libitum* basis. The houses were fumigated with formaldehyde gas after placement of the bedding material, 5 days before the arrival of the chicks. The temperature and ventilation of each of the facilities were closely monitored and regulated through the combined use of heating lamps and electrical fans. The temperature was initially kept at approximately 32 - 34ºC for the first two days after which it was gradually reduced by 2.8ºC per week. A lighting programme consisting of 23 hours light and 1-hour darkness was employed.

3.1.6) Diets: The chicks were reared on a 2 phase diet. A starter diet was fed from day 1 to 7 followed by a grower diet from day 8 to 21. The composition of the diets are given in Table 3.1 and Table 3.2, respectively. No coccidiostats or antibiotic growth promoters were included in the diets. All feed was irradiated after mixing with 5kGy (Isotron South Africa, Kempton Park) to prevent Salmonella contamination of chicks via the feed. The feed was formulated using Format Software (Format International, UK).

Table 3.1. Raw material composition and nutrient levels of the starter diet

Ingredient	**% Inclusion**
Yellow maize	59.6
Soya oil cake meal	26.4
Fish meal	11.0
Monocalcium phosphate	1.29
Limestone	1.07
Premix	0.50
Salt	0.15
Calculated Nutrient Levels	**g/kg**
Metabolisable energy	12.7 MJ/kg
Crude protein	243
Lysine	14.7
Methionine	4.80
Calcium	11.0
Available phosphorous	5.10
Sodium	1.66
Fat	38.8
Fibre	26.9

3.1.7) ***Measurements, sampling and sample analysis*****:** Cloacal swabs from 2 birds per pen were tested weekly for the presence of Salmonella. The swabs were enriched in Rappaport-Vassiliadis broth incubated over-night and plated onto Rambach agar at the Department of Microbiology and Plant Pathology, University of Pretoria. On days 7 and 14 of the trial one chick per replicate was sacrificed by cervical dislocation, while the remaining 2 chicks of each replicate were sacrificed on day 21. The gastrointestinal tracts (GIT) of all of these birds were examined for intestinal lesions. Blood samples were collected at each slaughter and centrifuged to obtain the serum that was analysed for its biochemical profile at the Department of Clinical Pathology, Onderstepoort, University of Pretoria.

Table 3.2. Raw material composition and nutrient levels of the grower diet

Ingredient	**% Inclusion**
Yellow maize	64.8
Soya oil cake meal	15.7
Fish meal	10.0
Full fat Soya	6.74
Monocalcium phosphate	1.19
Limestone	0.84
Premix	0.50
Salt	0.19
Calculated Nutrient Levels	**g/kg**
Metabolisable energy	13.22 MJ/kg
Crude protein	215
Lysine	12.7
Methionine	4.37
Calcium	9.50
Available phosphorous	4.70
Sodium	1.70
Fat	50.0
Fibre	27.3

a) Intestinal damage: Immediately after sacrifice the duodenum, jejenum and ileum were opened by a longitudinal incision along the antimesenteric side and cleaned of their contents in saline solution. These were examined for lesions with the length and width of each lesion being measured using a binocular lens. The extent was measured using a modified version of the scale used by Villegas *et al.* (2001); i.e. 0 = absence of haemorrhage, 1 = slight haemorrhage, 2 = moderate haemorrhage and 3 = severe haemorrhage.

b) Organ weights: The weights of the heart, liver, spleen, duodenum, jejenum, ileum and caeca were recorded and expressed as a percentage of body weight at the time of slaughter.

c) Histological examination: The bursa of Fabricius, caeca, spleen and liver were sent for histological examination at the Pathology Laboratory of the Faculty of Veterinary Science, Onderstepoort, University of Pretoria. These were imbedded following standard imbedding practices and stained with Haematoxylin and Eosin (H&E).

d) Broiler performance: Live chick body weights and feed intake were recorded for each pen on a weekly basis. Feed wastage and spillage were kept to a minimum with frequent monitoring. Feed conversion ratios were calculated and mortality was recorded as it occurred.

e) Serum biochemical profile: Total Serum Protein (TSP), aspartate transaminase activity (AST) and albumin and globulin levels in the serum were determined by the Department of Clinical Pathology, Onderstepoort, University of Pretoria.

Serum albumin

Serum samples were collected for albumin analyses. Albumin concentration was measured on a TECHNICON RA-1000® system (Miles Inc., Diagnostics Division, Tarrytown, New York, USA) according to standard procedures, as explained in the Technicon RA Systems Manual (Method No. SM4-0131E94, May 1994). This albumin method is based on the work of Doumas *et al*. (1971) who automated the original manual method of Rodkey (1965).

Total Serum Protein (TSP) - Serum samples were collected for TSP analyses. TSP concentrations were measured on a TECHNICON RA-1000® system (Miles Inc., Diagnostics Division, Tarrytown, New York, USA) according to standard procedures, as explained in the Technicon RA Systems Manual (Method No. SM4-0147E94, May 1994). This total method is based on the work of Skeggs & Hochstrasser (1964) who automated the manual method of Weichselbaum (1946).

Globulin – Serum globulin values were calculated as the difference between TSP and albumin.

Aspartate Aminotransferase (AST) - Serum samples were collected for AST analyses. AST concentrations were measured on a TECHNICON RA-1000® system (Miles Inc., Diagnostics Division, Tarrytown, New York, USA) according to standard procedures, as explained in the Technicon RA® Systems Manual (Method No. SM4-0137E94, May 1994). The Technicon RA® system AST method is based on work by Karmen (1955) who originated a procedure that coupled malate dehydrogenase and NADH to the aminotransferase reaction. Bergmeyer *et al*. (1978) modified this procedure to eliminate side reactions and to optimize substrate conditions.

3.1.8) Statistical Analysis: An analysis of variance with the GLM model (Statistical Analysis Systems, 2001) was used to determine the significance between different treatment levels and the interaction between treatments and levels. Means and standard deviations were calculated. Significance of difference (5%) between means was determined using Bonferroni's test.

3.2) Results

3.2.1) Organ weights

Exposure to Salmonella caused an increase in the weights of the duodenum (Table 3.3 and Table 3.4), jejunum (Table 3.7 and Table 3.8), ileum (Table 3.11 and Table 3.12), caeca (Table 3.15 and Table 3.16) and liver (Table 3.19 and Table 3.20) for both ages of the Salmonella inoculation. Heart weight was not affected by Salmonella exposure (Table 3.13 and Table 3.14).

Birds inoculated with Salmonella on day 4 had higher weights for the duodenum (Table 3.5 and Table 3.6), jejunum (Table 3.9 and Table 3.10), ileum (Table 3.13 and Table 3.14) and caeca (table 3.17 and Table 3.18). There were no significant differences in the liver (Table 3.21 and Table 3.22) and heart (Table 3.25 and Table 3.26) weights between the two ages of inoculation.

Cyclophosphamide appeared to have no effect on the organ weights of the broilers (Table 3.3, Table 3.4, Table 3.7, Table 3.8, Table 3.11, Table 3.12, Table 3.15, Table 3.16, Table 3.19, Table 3.20, Table 3.23 and Table 3.24)

Table 3.3 Duodenum weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	1.71 (±0.297)^{ab}	1.68 (±0.240)^{ab}
1 x 10^7	1.83 (±0.247)^{a}	1.79 (±0.299)^{a}
1 x 10^8	1.99 (±0.310)^{a}	2.08 (±0.573)^{a}
1 x 10^9	1.69 (±0.026)^{a}	2.13 (±0.006)^{a}
1 x 10^{10}	1.37 (±0.077)^{ab}	1.85 (±0.628)^{a}
Control (0 CFU/mL)	1.00 (±0.236)^{b}	0.98 (±0.244)^{b}

^{ab}Column means with same superscript do not differ significantly (P>0.0033)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.4 Duodenum weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+) and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	1.87 (±0.176)[a]	1.44 (±0.184)[ab]
1 x 10^7	1.53 (±0.149)[ab]	1.80 (±0.199)[a]
1 x 10^8	1.46 (±0.092)[ab]	1.55 (±0.093)[ab]
1 x 10^9	1.53 (±0.074)[ab]	1.60 (±0.299)[ab]
1 x 10^{10}	1.43 (±0.062)[ab]	1.53 (±0.147)[ab]
Control (0 CFU/mL)	1.00 (±0.236)[b]	0.98 (±0.244)[b]

[ab]Column means with same superscript do not differ significantly (P>0.0033)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.5 Duodenum weights expressed as percentage body weight, for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	1.71 (±0.297)	1.87 (±0.176)
1 x 10^7	1.83 (±0.247)	1.53 (±0.149)
1 x 10^8	1.99 (±0.310)[1]	1.46 (±0.092)[2]
1 x 10^9	1.69 (±0.026)	1.53 (±0.074)
1 x 10^{10}	1.37 (±0.077)	1.43 (±0.062)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.6 Duodenum weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	1.68 (±0.240)	1.44 (±0.184)
1 x 10^7	1.79 (±0.299)	1.80 (±0.199)
1 x 10^8	2.08 (±0.573)[1]	1.55 (±0.093)[2]
1 x 10^9	2.13 (±0.006)[1]	1.60 (±0.299)[2]
1 x 10^{10}	1.85 (±0.628)	1.53 (±0.147)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.7 Jejenum weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	1.97 (±0.459)	2.03 (±0.221)
1 x 10^7	2.37 (±0.235)	2.04 (±0.374)
1 x 10^8	2.27 (±0.276)	2.26 (±0.291)
1 x 10^9	2.11 (±0.298)	2.28 (±0.154)
1 x 10^{10}	1.77 (±0.085)[1]	2.34 (±0.669)[2]
Control (0 CFU/mL)	1.44 (±0.610)	1.30 (±0.426)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.8 Jejenum weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	1.70 (±0.182)	1.58 (±0.098)
1 x 10^7	1.94 (±0.157)	2.03 (±0.078)
1 x 10^8	1.78 (±0.112)	1.95 (±0.044)
1 x 10^9	1.71 (±0.219)	1.76 (±0.126)
1 x 10^{10}	1.50 (±0.041)	1.54 (±0.039)
Control (0 CFU/mL)	1.44 (±0.610)	1.30 (±0.426)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.9 Jejenum weights expressed as percentage body weight, for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	1.97 (±0.459)	1.70 (±0.182)
1 x 10^7	2.37 (±0.235)	1.94 (±0.157)
1 x 10^8	2.27 (±0.276)[1]	1.78 (±0.112)[2]
1 x 10^9	2.11 (±0.298)	1.71 (±0.219)
1 x 10^{10}	1.77 (±0.085)	1.50 (±0.041)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.10 Jejenum weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	2.03 (±0.221)	1.58 (±0.098)
1 x 10^7	2.04 (±0.374)	2.03 (±0.078)
1 x 10^8	2.26 (±0.291)	1.95 (±0.044)
1 x 10^9	2.28 (±0.154)^{1}	1.76 (±0.126)^{2}
1 x 10^{10}	2.34 (±0.669)^{1}	1.54 (±0.039)^{2}

^{12}Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.11 Ileum weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	**CY-**	**CY+**
1 x 10^6	1.66 (±0.442)^{abc}	1.61 (±0.145)^{ab}
1 x 10^7	1.79 (±0.411)^{ac}	1.72 (±0.399)^{a}
1 x 10^8	2.06 (±0.222)^{ac}	1.91 (±0.297)^{a}
1 x 10^9	1.81 (±0.062)^{ac}	2.02 (±0.035)^{a}
1 x 10^{10}	1.35 (±0.148)^{c}	1.83 (±0.454)^{a}
Control (0 CFU/mL)	1.08 (±0.331)^{b}	1.04 (±0.357)^{b}

^{abc}Column means with same superscript do not differ significantly (P>0.0033)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.12 Ileum weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	1.47 (±0.227)	1.28 (±0.192)[ab]
1 x 10^7	1.64 (±0.059)	1.71 (±0.224)[a]
1 x 10^8	1.50 (±0.076)	1.79 (±0.139)[a]
1 x 10^9	1.48 (±0.061)	1.78 (±0.424)[a]
1 x 10^{10}	1.33 (±0.062)	1.37 (±0.057)[ab]
Control (0 CFU/mL)	1.08 (±0.331)	1.04 (±0.357)[b]

[ab]Column means with same superscript do not differ significantly (P>0.0033)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.13 Ileum weights expressed as percentage body weight, for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	1.66 (±0.442)	1.47 (±0.227)
1 x 10^7	1.79 (±0.411)	1.64 (±0.059)
1 x 10^8	2.06 (±0.222)[1]	1.50 (±0.076)[2]
1 x 10^9	1.81 (±0.062)	1.48 (±0.061)
1 x 10^{10}	1.35 (±0.148)	1.33 (±0.062)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.14 Ileum weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	1.61 (±0.145)	1.28 (±0.192)
1 x 10^7	1.72 (±0.399)	1.71 (±0.224)
1 x 10^8	1.91 (±0.297)	1.79 (±0.139)
1 x 10^9	2.02 (±0.035)	1.78 (±0.424)
1 x 10^{10}	1.83 (±0.454)	1.37 (±0.057)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.15 Caeca weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	0.58 (±0.013)	0.74 (±0.015)[a]
1 x 10^7	0.70 (±0.090)	0.69 (±0.178)[ab]
1 x 10^8	0.57 (±0.088)	0.60 (±0.107)[ab]
1 x 10^9	0.65 (±0.080)	0.64 (±0.036)[ab]
1 x 10^{10}	0.59 (±0.101)	0.66 (±0.089)[ab]
Control (0 CFU/mL)	0.57 (±0.132)	0.47 (±0.143)[b]

[ab]Column means with same superscript do not differ significantly (P>0.0033)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.16 Caeca weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	0.73 (±0.121)[1]	0.57 (±0.083)[2]
1 x 10^7	0.57 (±0.191)	0.67 (±0.064)
1 x 10^8	0.56 (±0.061)	0.60 (±0.078)
1 x 10^9	0.54 (±0.056)	0.64 (±0.087)
1 x 10^{10}	0.66 (±0.058)	0.56 (±0.023)
Control (0 CFU/mL)	0.57 (±0.132)	0.47 (±0.143)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.17 Caeca weights expressed as percentage body weight, for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.58 (±0.013)	0.73 (±0.121)
1 x 10^7	0.70 (±0.090)	0.57 (±0.191)
1 x 10^8	0.57 (±0.088)	0.56 (±0.061)
1 x 10^9	0.65 (±0.080)	0.54 (±0.056)
1 x 10^{10}	0.59 (±0.101)	0.66 (±0.058)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.18 Caeca weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.74 (±0.015)[1]	0.57 (±0.083)[2]
1 x 10^7	0.69 (±0.178)	0.67 (±0.064)
1 x 10^8	0.60 (±0.107)	0.60 (±0.078)
1 x 10^9	0.64 (±0.036)	0.64 (±0.087)
1 x 10^{10}	0.66 (±0.089)	0.56 (±0.023)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.19 Liver weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	0.04 (±0.006)	0.05 (±0.007)[ab]
1 x 10^7	0.05 (±0.010)	0.05 (±0.022)[ab]
1 x 10^8	0.04 (±0.014)	0.04 (±0.004)[ab]
1 x 10^9	0.05 (±0.005)	0.06 (±0.010)[a]
1 x 10^{10}	0.05 (±0.018)	0.04 (±0.010)[ab]
Control (0 CFU/mL)	0.03 (±0.009)	0.03 (±0.006)[b]

[ab]Column means with same superscript do not differ significantly (P>0.0033)

*Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.20 Liver weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	0.07 (±0.012)^{ac1}	0.05 (±0.006)^{2}
1 x 10^7	0.04 (±0.001)^{abc}	0.04 (±0.011)
1 x 10^8	0.04 (±0.003)^{bc}	0.04 (±0.005)
1 x 10^9	0.04 (±0.004)^{abc}	0.04 (±0.005)
1 x 10^{10}	0.06 (±0.003)^{c}	0.05 (±0.006)
Control (0 CFU/mL)	0.03 (±0.009)^{b}	0.03 (±0.006)

^{abc}Column means with same superscript do not differ significantly (P>0.0033)

^{12}Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.21 Liver weights expressed as percentage body weight, for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.04 (±0.006)^{1}	0.07 (±0.012)^{2}
1 x 10^7	0.05 (±0.010)	0.04 (±0.001)
1 x 10^8	0.04 (±0.014)	0.04 (±0.003)
1 x 10^9	0.05 (±0.005)	0.04 (±0.004)
1 x 10^{10}	0.05 (±0.018)	0.06 (±0.003)

^{12}Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.22 Liver weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.05 (±0.007)	0.05 (±0.006)
1 x 10^7	0.05 (±0.022)	0.04 (±0.011)
1 x 10^8	0.04 (±0.004)	0.04 (±0.005)
1 x 10^9	0.06 (±0.010)	0.04 (±0.005)
1 x 10^{10}	0.04 (±0.010)	0.05 (±0.006)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.23 Heart weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	0.01 (±0.001)	0.01 (±0.001)
1 x 10^7	0.01 (±0.001)	0.01 (±0.006)
1 x 10^8	0.01 (±0.003)	0.01 (±0.001)
1 x 10^9	0.01 (±0.002)	0.01 (±0.001)
1 x 10^{10}	0.01 (±0.002)	0.01 (±0.002)
Control (0 CFU/mL)	0.01 (±0.002)	0.01 (±0.001)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.24 Heart weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	0.01 (±0.001)	0.01 (±0.001)
1 x 10^7	0.01 (±0.005)[1]	0.01 (±0.001)[2]
1 x 10^8	0.01 (±0.001)	0.01 (±0.002)
1 x 10^9	0.01 (±0.002)	0.01 (±0.002)
1 x 10^{10}	0.01 (±0.001)	0.01 (±0.001)
Control (0 CFU/mL)	0.01 (±0.002)	0.01 (±0.001)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.25 Heart weights expressed as percentage body weight, for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.01 (±0.001)	0.01 (±0.001)
1 x 10^7	0.01 (±0.001)	0.01 (±0.005)
1 x 10^8	0.01 (±0.003)	0.01 (±0.001)
1 x 10^9	0.01 (±0.002)	0.01 (±0.002)
1 x 10^{10}	0.01 (±0.002)	0.01 (±0.001)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.26 Heart weights expressed as percentage body weight, for broilers exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.01 (±0.001)	0.01 (±0.001)
1 x 10^7	0.01 (±0.006)[1]	0.01 (±0.001)[2]
1 x 10^8	0.01 (±0.001)	0.01 (±0.002)
1 x 10^9	0.01 (±0.001)	0.01 (±0.002)
1 x 10^{10}	0.01 (±0.002)	0.01 (±0.001)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

3.2.2) Broiler performance

At hatch there were no significant differences in the body weights of the chicks, showing that the chicks were randomly placed with similar body weights at the beginning of the trial (Tables 3.27 - 3.30).

Groups challenged with Salmonella had lower BW (Table 3.31, Table 3.32, Table 3.35, Table 3.36, Table 3.39 and Table 3.40) and lower ADG (Table 3.59, Table 3.60, Table 3.63, Table 3.64, Table 3.67, Table 3.68, Table 3.71 and Table 3.72) than the control birds throughout the trial. Groups challenged with Salmonella had lower FI than the control birds for the first 2 weeks of the trial (Table 3.43, Table 3.44, Table 3.47 and Table 3.48) and higher FI in the third week of the trial as well as for the cumulative FI (Table 3.51, Table 3.52, Table 3.55 and Table 3.56). Groups challenged with Salmonella had higher FCR than the control birds for week 1, week 3 and cumulative FCR (Table 3.75, Table 3.76, Table 3.83, Table 3.84, Table 3.87 and Table 3.88) and lower FCR for the second week of the trial (Table 3.79 and Table 3.80).

Birds challenged with Salmonella on day 4 of the trial had higher BW (Table 3.33 and Table 3.34), lower FI (Table 3.45 and Table 3.46), higher ADG (Table 3.61 and Table 3.62) and lower FCR (Table 3.77 and Table 3.78) than those challenged on day 7 during the first week of the trial.

In week two of the trial, the birds that were challenged on day 4 had lower BW (Table 3.37), lower FI (Table 3.49), lower ADG (Table 3.65) and lower FCR (Table 3.81) than the birds challenged on day 7 for the groups that did not receive CY, while in the groups that did receive CY the birds challenged on day 4 with Salmonella higher BW (Table 3.38), higher FI (Table 3.50), higher ADG (Table 3.66) and higher FCR (Table 3.82) than the birds challenged on day 7 of the trial.

In week three of the trial, the birds that were challenged with Salmonella on day 4 had lower BW (Table 3.41 and Table 3.42), higher FI (Table 3.53 and Table 3.54), lower ADG (Table 3.69 and Table 3.70) and higher FCR (Table 3.85 and Table 3.86) than the birds challenged on day 7 of the trial.

Birds challenged on day 4 of the trial with Salmonella had a lower cumulative FCR (Table 3.57 and Table 3.58), a lower cumulative ADG (Table 3.73 and Table 3.74) and a higher cumulative FCR (Table 3.89 and Table 3.90) than the birds that were challenged on day 7. Exposure to CY did not significantly affect broiler performance.

Table 3.27 Body weight (BW) at hatch for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	46.26 (±0.901)	44.65 (±1.552)
1 x 10^7	43.88 (±2.801)	44.01 (±0.890)
1 x 10^8	47.69 (±1.688)[1]	44.45 (±2.240)[2]
1 x 10^9	46.69 (±1.548)	45.00 (±0.740)
1 x 10^{10}	45.72 (±1.289)	45.34 (±0.895)
Control (0 CFU/mL)	46.35 (±0.823)	44.48 (±1.032)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.28 Body weight (BW) at hatch for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	44.68 (±1.040)	45.38 (±0.958)
1 x 10^7	46.21 (±2.079)	45.83 (±1.213)
1 x 10^8	45.60 (±2.001)	45.63 (±0.592)
1 x 10^9	44.81 (±1.246)	44.55 (±2.588)
1 x 10^{10}	46.04 (±2.047)	45.24 (±0.511)
Control (0 CFU/mL)	46.35 (±0.823)	44.48 (±1.032)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.29 Body weight (BW) at hatch for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	46.26 (±0.901)	44.68 (±1.040)
1 x 10^7	43.88 (±2.801)	46.21 (±2.079)
1 x 10^8	47.69 (±1.688)	45.60 (±2.001)
1 x 10^9	46.69 (±1.548)	44.81 (±1.246)
1 x 10^{10}	45.72 (±1.289)	46.04 (±2.047)
Control (0 CFU/mL)	46.35 (±0.823)	46.35 (±0.823)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.30 Body weight (BW) at hatch for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	44.65 (±1.552)	45.38 (±0.958)
1 x 10^7	44.01 (±0.890)	45.83 (±1.213)
1 x 10^8	44.45 (±2.240)	45.63 (±0.592)
1 x 10^9	45.00 (±0.740)	44.55 (±2.588)
1 x 10^{10}	45.34 (±0.895)	45.24 (±0.511)
Control (0 CFU/mL)	44.48 (±1.032)	44.48 (±1.032)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.31 Body weight (BW) at day 7 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	**CY-**	**CY+**
1 x 10^6	72.61 (±21.217)^{a1}	100.06 (±6.392)^{2}
1 x 10^7	100.43 (±32.427)^{ab}	101.43 (±101.427)
1 x 10^8	89.49 (±13.929)^{ab}	91.78 (±19.318)
1 x 10^9	85.18 (±30.817)^{ab}	109.32 (±19.763)
1 x 10^{10}	96.77 (±41.097)^{ab}	103.65 (±5.522)
Control (0 CFU/mL)	124.04 (±11.476)^{b}	109.85 (±3.896)

^{ab}Column means with same superscript do not differ significantly (P>0.0033)

^{12}Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.32 Body weight (BW) at day 7 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	93.08 (±4.790)	112.8 (±10.34)
1 x 10^7	97.33 (±4.078)	84.28 (±23.92)
1 x 10^8	84.93 (±9.475)	91.64 (±4.183)
1 x 10^9	92.59 (±14.25)	87.93 (±30.71)
1 x 10^{10}	89.36 (±8.656)	108.81 (±44.07)
Control (0 CFU/mL)	124.0 (±11.48)	109.85 (±3.90)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.33 Body weight (BW) at day 7 for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	72.61 (±21.22)	93.08 (±4.790)
1 x 10^7	100.4 (±32.43)	97.33 (±4.078)
1 x 10^8	89.49 (±13.93)	84.93 (±9.475)
1 x 10^9	85.18 (±30.82)	92.59 (±14.25)
1 x 10^{10}	96.77 (±41.10)	89.36 (±8.656)
Control (0 CFU/mL)	124.0 (±11.48)	124.04(±11.48)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.34 Body weight (BW) at day 7 for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	100.06 (±6.392)	112.8 (±10.34)
1 x 10^7	101.4 (±101.43)	84.28 (±23.92)
1 x 10^8	91.78 (±19.32)	91.64 (±4.183)
1 x 10^9	109.3 (±19.76)	87.93 (±30.71)
1 x 10^{10}	103.7 (±5.522)	108.8 (±44.07)
Control (0 CFU/mL)	109.9 (±3.896)	109.9 (±3.896)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.35 Body weight (BW) at day 14 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	284.4 (±20.83)	337.0 (±61.37)
1 x 10^7	328.4 (±102.9)	310.1 (±45.21)
1 x 10^8	277.5 (±1.400)	316.7 (±46.88)
1 x 10^9	289.4 (±30.38)	328.0 (±31.87)
1 x 10^{10}	290.8 (±50.98)	211.4 (±150.9)
Control (0 CFU/mL)	355.7 (±16.33)	327.3 (±3.839)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.36 Body weight (BW) at day 14 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	306.5 (±18.49)	386.3 (±32.72)
1 x 10^7	314.9 (±7.577)	276.4 (±78.28)
1 x 10^8	290.8 (±27.14)	311.6 (±7.915)
1 x 10^9	298.2 (±69.57)	308.4 (±55.25)
1 x 10^{10}	302.7 (±31.82)	341.8 (±50.45)
Control (0 CFU/mL)	355.7 (±16.33)	327.3 (±3.839)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.37 Body weight (BW) at day 14 for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	284.4 (±20.83)	306.5 (±18.49)
1 x 10^7	328.4 (±102.9)	314.9 (±7.577)
1 x 10^8	277.5 (±1.400)	290.8 (±27.14)
1 x 10^9	289.4 (±30.38)	298.2 (±69.57)
1 x 10^{10}	290.8 (±50.98)	302.7 (±31.82)
Control (0 CFU/mL)	355.7 (±16.33)	355.7 (±16.33)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.38 Body weight (BW) at day 14 for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	337.0 (±61.37)	386.3 (±32.72)
1 x 10^7	310.1 (±45.21)	276.4 (±78.28)
1 x 10^8	316.7 (±46.88)	311.6 (±7.915)
1 x 10^9	328.0 (±31.87)	308.4 (±55.25)
1 x 10^{10}	211.4 (±150.9)	341.8 (±50.45)
Control (0 CFU/mL)	327.3 (±3.839)	327.3 (±3.839)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.39 Body weight (BW) at day 21 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	**CY-**	**CY+**
1 x 10^6	511.8 (±70.30)^{a}	615.4 (±109.2)^{a}
1 x 10^7	382.9 (±42.79)^{a1}	601.3 (±97.75)^{a2}
1 x 10^8	464.2 (±51.07)^{a}	475.8 (±58.49)^{a}
1 x 10^9	460.9 (±2.277)^{a}	376.4 (±20.78)^{b}
1 x 10^{10}	542.6 (±109.8)^{ab}	606.8 (±72.88)^{a}
Control (0 CFU/mL)	769.5 (±41.86)^{b}	782.7 (±27.14)^{a}

^{ab}Column means with same superscript do not differ significantly (P>0.0033)

^{12}Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.40 Body weight (BW) at day 21 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	629.4 (±51.67)^{ab}	714.0 (±18.58)^{ab}
1 x 10^7	672.4 (±98.99)^{ab}	561.3 (±202.3)^{a}
1 x 10^8	592.2 (±65.25)^{ab}	606.8 (±30.48)^{ab}
1 x 10^9	532.8 (±132.7)^{a}	569.7 (±110.9)^{ab}
1 x 10^{10}	608.1 (±37.82)^{ab}	685.4 (±114.4)^{ab}
Control (0 CFU/mL)	769.5 (±41.86)^{b}	782.7 (±27.14)^{b}

^{ab}Column means with same superscript do not differ significantly (P>0.0033)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.41 Body weight (BW) at day 21 for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	511.78 (±70.30)	629.4 (±109.2)
1 x 10^7	382.9 (±42.79)^{1}	672.4 (±97.75)^{2}
1 x 10^8	464.2 (±51.07)	592.2 (±58.49)
1 x 10^9	460.9 (±2.277)	532.8 (±20.78)
1 x 10^{10}	542.6 (±109.8)	608.1 (±72.88)
Control (0 CFU/mL)	769.5 (±41.86)	769.5 (±27.14)

^{12}Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.42 Body weight (BW) at day 21 for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	615.4 (±109.2)	714.0 (±18.58)
1 x 10^7	601.3 (±97.75)	561.3 (±202.3)
1 x 10^8	475.8 (±58.49)	606.8 (±30.48)
1 x 10^9	376.4 (±20.78)	569.7 (±110.9)
1 x 10^{10}	606.8 (±72.88)	685.4 (±114.4)
Control (0 CFU/mL)	782.7 (±27.14)	782.7 (±27.14)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.43 Feed intake (FI) at day 7 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	54.39 (±22.49)[a]	78.47 (±8.396)
1 x 10^7	79.50 (±37.78)[ab]	83.18 (±18.49)
1 x 10^8	66.83 (±15.74)[ab]	77.15 (±21.01)
1 x 10^9	62.65 (±35.73)[ab]	78.39 (±16.55)
1 x 10^{10}	77.01 (±39.93)[ab]	94.15 (±10.96)
Control (0 CFU/mL)	109.1 (±10.41)[b]	85.03 (±18.38)

[ab]Column means with same superscript do not differ significantly (P>0.0033)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.44 Feed intake (FI) at day 7 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	58.93 (±20.05)[a]	66.36 (±30.51)
1 x 10^7	79.55 (±4.32)[ab]	87.40 (±12.26)
1 x 10^8	73.58 (±6.52)[ab]	75.43 (±7.072)
1 x 10^9	80.27 (±19.47)[ab]	75.83 (±32.40)
1 x 10^{10}	93.47 (±18.08)[ab]	101.2 (±10.44)
Control (0 CFU/mL)	109.1 (±10.41)[b]	85.03 (±18.38)

[ab]Column means with same superscript do not differ significantly (P>0.0033)

*Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.45 Feed intake(FI) at day 7 for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	54.39 (±22.49)	58.93 (±20.05)
1 x 10^7	79.50 (±37.78)	79.55 (±4.32)
1 x 10^8	66.83 (±15.74)	73.58 (±6.52)
1 x 10^9	62.65 (±35.73)	80.27 (±19.47)
1 x 10^{10}	77.01 (±39.93)	93.47 (±18.08)
Control (0 CFU/mL)	109.1 (±10.41)	109.1 (±10.41)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.46 Feed intake (FI) at day 7 for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	78.47 (±8.396)	66.36 (±30.51)
1 x 10^7	83.18 (±18.49)	87.40 (±12.26)
1 x 10^8	77.15 (±21.01)	75.43 (±7.072)
1 x 10^9	78.39 (±16.55)	75.83 (±32.40)
1 x 10^{10}	94.15 (±10.96)	101.2 (±10.44)
Control (0 CFU/mL)	85.03 (±18.38)	85.03 (±18.38)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.47 Feed intake (FI) at day 14 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	**CY-**	**CY+**
1 x 10^6	185.7 (±32.93)^a	309.2 (±31.36)
1 x 10^7	291.7 (±128.1)^{ab}	242.3 (±19.79)
1 x 10^8	251.7 (±53.20)^{ab}	281.3 (±53.51)
1 x 10^9	209.0 (±103.0)^{ab}	262.9 (±37.08)
1 x 10^{10}	254.1 (±61.74)^{ab}	280.3 (±64.11)
Control (0 CFU/mL)	337.4 (±22.85)^b	314.2 (±12.19)

^{ab}Column means with same superscript do not differ significantly (P>0.0033)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.48 Feed intake (FI) at day 14 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	**CY-**	**CY+**
1 x 10^6	310.6 (±45.8906)	306.7 (±18.02)
1 x 10^7	244.7 (±3.502)	221.4 (±93.29)
1 x 10^8	237.4 (±39.33)	277.8 (±31.41)
1 x 10^9	276.0 (±81.37)	239.6 (±77.90)
1 x 10^{10}	271.1 (±48.98)	289.7 (±30.53)
Control (0 CFU/mL)	337.4 (±22.85)	314.2 (±12.19)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.49 Feed intake (FI) at day 14 for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	185.7 (±32.93)[1]	310.6 (±45.89)[2]
1 x 10^7	291.7 (±128.1)	244.7 (±3.50)
1 x 10^8	251.7 (±53.20)	237.4 (±39.33)
1 x 10^9	209.0 (±103.0)	276.0 (±81.37)
1 x 10^{10}	254.1 (±61.74)	271.1 (±48.98)
Control (0 CFU/mL)	337.4 (±22.85)	337.4 (±22.85)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.50 Feed intake (FI) at day 14 for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	309.2 (±31.36)	306.7 (±18.02)
1 x 10^7	242.3 (±19.79)	221.4 (±93.29)
1 x 10^8	281.3 (±53.51)	277.8 (±31.41)
1 x 10^9	262.9 (±37.08)	239.6 (±77.90)
1 x 10^{10}	280.3 (±64.11)	289.7 (±30.53)
Control (0 CFU/mL)	314.2 (±12.19)	314.2 (±12.19)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.51 Feed intake (FI) at day 21 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	**CY-**	**CY+**
1 x 10^6	654.0 (±148.1)	713.7 (±59.34)
1 x 10^7	675.4 (±19.20)	731.5 (±7.130)
1 x 10^8	696.7 (±122.5)	723.8 (±123.6)
1 x 10^9	667.2 (±54.94)	651.7 (±32.07)
1 x 10^{10}	687.7 (±80.92)	718.8 (±30.40)
Control (0 CFU/mL)	666.0 (±35.86)	668.6 (±20.76)

*Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.52 Feed intake (FI) at day 21 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	756.8 (±135.0)	739.7 (±3.329)
1 x 10^7	686.1 (±32.81)	594.2 (±165.6)
1 x 10^8	620.7 (±96.59)	651.4 (±31.95)
1 x 10^9	590.2 (±103.9)	658.4 (±126.7)
1 x 10^{10}	683.6 (±61.56)	677.0 (±72.11)
Control (0 CFU/mL)	666.0 (±35.86)	668.6 (±20.76)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.53 Feed intake (FI) at day 21 for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	654.0 (±148.1)	756.8 (±135.0)
1 x 10^7	675.4 (±19.20)	686.1 (±32.81)
1 x 10^8	696.7 (±122.5)	620.7 (±96.59)
1 x 10^9	667.2 (±54.94)	590.2 (±103.9)
1 x 10^{10}	687.7 (±80.92)	683.6 (±61.56)
Control (0 CFU/mL)	666.0 (±35.86)	666.0 (±35.86)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.54 Feed intake (FI) at day 21 for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	713.7 (±59.34)	739.7 (±3.329)
1 x 10^7	731.5 (±7.130)	594.2 (±165.6)
1 x 10^8	723.8 (±123.6)	651.4 (±31.95)
1 x 10^9	651.7 (±32.07)	658.4 (±126.7)
1 x 10^{10}	718.8 (±30.40)	677.0 (±72.11)
Control (0 CFU/mL)	668.6 (±20.76)	668.6 (±20.76)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.55 Cumulative feed intake (FI) for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	654.0 (±148.1)	756.8 (±135.0)
1 x 10^7	675.4 (±19.11)	686.1 (±32.81)
1 x 10^8	696.7 (±122.5)	620.7 (±96.59)
1 x 10^9	667.2 (±54.94)	590.2 (±103.9)
1 x 10^{10}	687.7 (±80.92)	683.6 (±61.56)
Control (0 CFU/mL)	666.0 (±35.86)	666.0 (±35.86)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.56 Cumulative feed intake (FI) for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	713.7 (±59.34)	739.7 (±3.329)
1 x 10^7	731.5 (±7.130)	594.2 (±165.6)
1 x 10^8	723.8 (±123.6)	651.4 (±31.95)
1 x 10^9	651.7 (±32.07)	658.9 (±126.7)
1 x 10^{10}	718.8 (±30.40)	677.0 (±72.11)
Control (0 CFU/mL)	668.6 (±20.76)	668.6 (±20.76)

*Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.57 Cumulative feed intake (FI) for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	654.0 (±148.1)	713.7 (±59.34)
1 x 10^7	675.4 (±19.11)	731.5 (±7.130)
1 x 10^8	696.7 (±122.5)	723.8 (±123.6)
1 x 10^9	667.2 (±54.94)	651.7 (±32.07)
1 x 10^{10}	687.7 (±80.92)	718.8 (±30.40)
Control (0 CFU/mL)	666.0 (±35.86)	668.6 (±20.76)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.58 Cumulative feed intake (FI) for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	756.8 (±135.0)	739.7 (±3.329)
1 x 10^7	686.1 (±32.81)	594.2 (±165.6)
1 x 10^8	620.7 (±96.59)	651.4 (±31.95)
1 x 10^9	590.2 (±103.9)	658.9 (±126.7)
1 x 10^{10}	683.6 (±61.56)	677.0 (±72.11)
Control (0 CFU/mL)	666.0 (±35.86)	668.6 (±20.76)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.59 Average daily gain (ADG) at day 7 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	3.76 (±2.991)^a	7.92 (±1.015)
1 x 10^7	8.08 (±4.457)^{ab}	8.20 (±2.312)
1 x 10^8	5.97 (±2.038)^{ab}	6.76 (±2.596)
1 x 10^9	5.50 (±4.219)^{ab}	9.19 (±2.805)
1 x 10^{10}	7.29 (±4.399)^{ab}	8.33 (±0.910)
Control (0 CFU/mL)	11.10 (±1.550)^b	9.34 (±0.657)

^{ab}Column means with same superscript do not differ significantly (P>0.0033)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.60 Average daily gain (ADG) at day 7 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	6.91 (±6.084)	9.64 (±1.390)
1 x 10^7	7.30 (±0.811)	5.49 (±9.867)
1 x 10^8	5.62 (±1.253)	6.57 (±0.668)
1 x 10^9	6.83 (±3.252)	6.20 (±4.330)
1 x 10^{10}	6.19 (±1.461)	9.08 (±13.146)
Control (0 CFU/mL)	11.10 (±1.550)	9.34 (±0.657)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.61 Average daily gain (ADG) at day 7 for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	3.76 (±2.991)	6.91 (±6.084)
1 x 10^7	8.08 (±4.457)	7.30 (±0.811)
1 x 10^8	5.97 (±2.038)	5.62 (±1.253)
1 x 10^9	5.50 (±4.219)	6.83 (±3.252)
1 x 10^{10}	7.29 (±4.399)	6.19 (±1.461)
Control (0 CFU/mL)	11.10 (±1.550)	11.10 (±1.550)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.62 Average daily gain (ADG) at day 7 for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	7.92 (±1.015)	9.64 (±1.390)
1 x 10^7	8.20 (±2.312)	5.49 (±9.867)
1 x 10^8	6.76 (±2.596)	6.57 (±0.668)
1 x 10^9	9.19 (±2.805)	6.20 (±4.330)
1 x 10^{10}	8.33 (±0.910)	9.08 (±13.146)
Control (0 CFU/mL)	9.34 (±0.657)	9.34 (±0.657)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.63 Average daily gain (ADG) at day 14 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	**CY-**	**CY+**
1 x 10^6	17.01 (±1.440)	20.88 (±4.325)
1 x 10^7	20.32 (±7.262)	19.01 (±3.169)
1 x 10^8	16.42 (±0.065)	19.45 (±3.203)
1 x 10^9	17.34 (±2.217)	20.21 (±2.295)
1 x 10^{10}	17.51 (±3.673)	11.86 (±1.548)
Control (0 CFU/mL)	22.10 (±1.118)	20.20 (±0.341)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.64 Average daily gain (ADG) at day 14 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	18.70 (±2.978)	24.35 (±2.831)
1 x 10^7	19.19 (±0.542)	16.47 (±8.599)
1 x 10^8	17.51 (±1.854)	19.00 (±0.548)
1 x 10^9	18.10 (±3.736)	18.85 (±3.916)
1 x 10^{10}	18.33 (±1.332)	21.18 (±5.863)
Control (0 CFU/mL)	22.10 (±1.118)	20.20 (±0.341)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.65 Average daily gain (ADG) at day 14 for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	17.01 (±1.440)	18.70 (±2.978)
1 x 10^7	20.32 (±7.262)	19.19 (±0.542)
1 x 10^8	16.42 (±0.065)	17.51 (±1.854)
1 x 10^9	17.34 (±2.217)	18.10 (±3.736)
1 x 10^{10}	17.51 (±3.673)	18.33 (±1.332)
Control (0 CFU/mL)	22.10 (±1.118)	22.10 (±1.118)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.66 Average daily gain (ADG) at day 14 for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	20.88 (±4.325)	24.35 (±2.831)
1 x 10^7	19.01 (±3.169)	16.47 (±8.599)
1 x 10^8	19.45 (±3.203)	19.00 (±0.548)
1 x 10^9	20.21 (±2.295)	18.85 (±3.916)
1 x 10^{10}	11.86 (±1.548)	21.18 (±5.863)
Control (0 CFU/mL)	20.20 (±0.341)	20.20 (±0.341)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.67 Average daily gain (ADG) at day 21 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	22.17 (±3.305)^{a}	27.18 (±5.216)^{ac}
1 x 10^7	16.14 (±1.849)^{a1}	26.54 (±4.612)^{ac2}
1 x 10^8	19.83 (±2.543)^{a}	20.54 (±2.872)^{ab}
1 x 10^9	19.72 (±0.139)^{a}	15.78 (±0.971)^{b}
1 x 10^{10}	23.66 (±5.249)^{ab}	26.73 (±3.468)^{a}
Control (0 CFU/mL)	34.44 (±2.018)^{b}	35.15 (±1.294)^{c}

^{abc}Column means with same superscript do not differ significantly (P>0.0033)

^{12}Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.68 Average daily gain (ADG) at day 21 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	27.84 (±3.250)^{ab}	31.84 (±0.728)^{ab}
1 x 10^7	29.82 (±4.764)^{ab}	24.55 (±11.48)^{a}
1 x 10^8	26.03 (±3.032)^{ab}	26.72 (±1.431)^{ab}
1 x 10^9	23.24 (±7.402)^{a}	25.01 (±5.255)^{ab}
1 x 10^{10}	26.77 (±1.174)^{ab}	30.48 (±6.953)^{ab}
Control (0 CFU/mL)	34.44 (±2.018)^{b}	35.15 (±1.294)^{b}

^{ab}Column means with same superscript do not differ significantly (P>0.0033)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.69 Average daily gain (ADG) at day 21 for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	22.17 (±3.305)	27.84 (±3.250)
1 x 10^7	16.14 (±1.849)[1]	29.82 (±4.764)[2]
1 x 10^8	19.83 (±2.543)	26.03 (±3.032)
1 x 10^9	19.72 (±0.139)	23.24 (±7.402)
1 x 10^{10}	23.66 (±5.249)	26.77 (±1.174)
Control (0 CFU/mL)	34.44 (±2.018)	34.44 (±2.018)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.70 Average daily gain (ADG) at day 21 for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	27.18 (±5.216)	31.84 (±0.728)
1 x 10^7	26.54 (±4.612)	24.55 (±11.48)
1 x 10^8	20.54 (±2.872)	26.72 (±1.431)
1 x 10^9	15.78 (±0.971)[1]	25.01 (±5.255)[2]
1 x 10^{10}	26.73 (±3.468)	30.48 (±6.953)
Control (0 CFU/mL)	35.15 (±1.294)	35.15 (±1.294)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.71 Cumulative average daily gain (ADG) for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	16.63 (±2.479)^{a}	20.38 (±3.912)^{ac}
1 x 10^7	12.11 (±1.390)^{a1}	19.90 (±3.460)^{ac2}
1 x 10^8	14.88 (±1.906)^{a}	15.40 (±2.153)^{ab}
1 x 10^9	14.79 (±0.105)^{a}	11.83 (±0.728)^{b}
1 x 10^{10}	17.75 (±3.936)^{ab}	20.05 (±2.601)^{ac}
Control (0 CFU/mL)	25.83 (±1.514)^{b}	26.36 (±0.971)^{c}

^{abc}Column means with same superscript do not differ significantly (P>0.0033)

^{12}Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.72 Cumulative average daily gain (ADG) for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	20.88 (±1.814)^{ab}	23.88 (±0.634)^{ab}
1 x 10^7	22.36 (±3.592)^{ab}	18.41 (±7.201)^{a}
1 x 10^8	19.52 (±2.350)^{ab}	20.04 (±1.095)^{ab}
1 x 10^9	17.43 (±4.747)^{a}	18.75 (±3.873)^{ab}
1 x 10^{10}	20.07 (±1.277)^{ab}	22.86 (±4.111)^{ab}
Control (0 CFU/mL)	25.83 (±1.514)^{b}	26.36 (±0.971)^{b}

^{ab}Column means with same superscript do not differ significantly (P>0.0033)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.73 Cumulative average daily gain (ADG) for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	16.63 (±2.479)	20.88 (±1.814)
1 x 10^7	12.11 (±1.390)[1]	22.36 (±3.592)[2]
1 x 10^8	14.88 (±1.906)	19.52 (±2.350)
1 x 10^9	14.79 (±0.105)	17.43 (±4.747)
1 x 10^{10}	17.75 (±3.936)	20.07 (±1.277)
Control (0 CFU/mL)	25.83 (±1.514)	25.83 (±1.514)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.74 Cumulative average daily gain (ADG) for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	20.38 (±3.912)	23.88 (±0.634)
1 x 10^7	19.90 (±3.460)	18.41 (±7.201)
1 x 10^8	15.40 (±2.153)	20.04 (±1.095)
1 x 10^9	11.83 (±0.728)[1]	18.75 (±3.873)[2]
1 x 10^{10}	20.05 (±2.601)	22.86 (±4.111)
Control (0 CFU/mL)	26.36 (±0.971)	26.36 (±0.971)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.75 Feed conversion ratio (FCR) at day 7 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	**CY-**	**CY+**
1 x 10^6	0.73 (±4.964)	0.79 (±0.178)
1 x 10^7	0.78 (±1.181)	0.82 (±0.176)
1 x 10^8	0.75 (±0.282)	0.83 (±0.255)
1 x 10^9	0.73 (±1.146)	0.72 (±0.316)
1 x 10^{10}	0.78 (±2.362)	0.91 (±0.044)
Control (0 CFU/mL)	0.88 (±0.060)	0.77 (±0.256)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.76 Feed conversion ratio (FCR) at day 7 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	0.63 (±0.423)[a]	0.58 (±0.439)[a]
1 x 10^7	0.82 (±0.100)[ab1]	1.12 (±3.507)[b2]
1 x 10^8	0.87 (±0.320)[ab]	0.82 (±0.069)[ab]
1 x 10^9	0.86 (±0.164)[ab]	0.84 (±1.044)[ab]
1 x 10^{10}	1.04 (±0.195)[ab]	0.94 (±8.900)[ab]
Control (0 CFU/mL)	0.88 (±0.060)[b]	0.77 (±0.256)[ab]

[ab]Column means with same superscript do not differ significantly (P>0.0033)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.77 Feed conversion ratio (FCR) at day 7 for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.73 (±4.964)	0.63 (±0.423)
1 x 10^7	0.78 (±1.181)	0.82 (±0.100)
1 x 10^8	0.75 (±0.282)	0.87 (±0.320)
1 x 10^9	0.73 (±1.146)	0.86 (±0.164)
1 x 10^{10}	0.78 (±2.362)	1.04 (±0.195)
Control (0 CFU/mL)	0.88 (±0.060)	0.88 (±0.060)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.78 Feed conversion ratio (FCR) at day 7 for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.79 (±0.178)	0.58 (±0.439)
1 x 10^7	0.82 (±0.176)[1]	1.12 (±3.507)[2]
1 x 10^8	0.83 (±0.255)	0.82 (±0.069)
1 x 10^9	0.72 (±0.316)	0.84 (±1.044)
1 x 10^{10}	0.91 (±0.044)	0.94 (±8.900)
Control (0 CFU/mL)	0.77 (±0.256)	0.77 (±0.256)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.79 Feed conversion ratio (FCR) at day 14 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	**CY-**	**CY+**
1 x 10^6	0.66 (±0.132)	0.95 (±0.464)[ab]
1 x 10^7	0.90 (±0.440)	0.79 (±0.088)[a]
1 x 10^8	0.91 (±0.345)	0.89 (±0.290)[a]
1 x 10^9	0.71 (±0.414)	0.80 (±0.084)[a]
1 x 10^{10}	0.87 (±0.198)[1]	3.08 (±0.302)[b2]
Control (0 CFU/mL)	0.95 (±0.069)	0.96 (±0.050)[ab]

[ab]Column means with same superscript do not differ significantly (P>0.0033)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.80 Feed conversion ratio (FCR) at day 14 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	1.01 (±0.166)	0.80 (±0.207)
1 x 10^7	0.78 (±0.037)	0.77 (±0.211)
1 x 10^8	0.81 (±0.091)	0.89 (±0.163)
1 x 10^9	0.92 (±0.099)	0.76 (±0.249)
1 x 10^{10}	0.89 (±0.068)	0.85 (±0.018)
Control (0 CFU/mL)	0.95 (±0.069)	0.96 (±0.050)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.81 Feed conversion ratio (FCR) at day 14 for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.66 (±0.132)	1.01 (±0.166)
1 x 10^7	0.90 (±0.440)	0.78 (±0.037)
1 x 10^8	0.91 (±0.345)	0.81 (±0.091)
1 x 10^9	0.71 (±0.414)	0.92 (±0.099)
1 x 10^{10}	0.87 (±0.198)	0.89 (±0.068)
Control (0 CFU/mL)	0.95 (±0.069)	0.95 (±0.069)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.82 Feed conversion ratio (FCR) at day 14 for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.95 (±0.464)	0.80 (±0.207)
1 x 10^7	0.79 (±0.088)	0.77 (±0.211)
1 x 10^8	0.89 (±0.290)	0.89 (±0.163)
1 x 10^9	0.80 (±0.084)	0.76 (±0.249)
1 x 10^{10}	3.08 (±0.302)[1]	0.85 (±0.018)[2]
Control (0 CFU/mL)	0.96 (±0.050)	0.96 (±0.050)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.83 Feed conversion ratio (FCR) at day 21 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	1.27 (±0.268)^{a}	1.18 (±0.598)^{ac}
1 x 10^7	1.78 (±6.399)^{c1}	1.24 (±0.517)^{a2}
1 x 10^8	1.50 (±0.409)^{ac}	1.54 (±4.056)^{ab}
1 x 10^9	1.45 (±0.971)^{a1}	1.74 (±0.658)^{b2}
1 x 10^{10}	1.28 (±0.325)^{a}	1.20 (±0.509)^{ac}
Control (0 CFU/mL)	0.87 (±0.114)^{b}	0.86 (±0.128)^{c}

^{abc}Column means with same superscript do not differ significantly (P>0.0033)

^{12}Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.84 Feed conversion ratio (FCR) at day 21 for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	1.20 (±0.405)	1.04 (±0.351)
1 x 10^7	1.03 (±0.413)	1.09 (±0.543)
1 x 10^8	1.05 (±0.041)	1.07 (±0.064)
1 x 10^9	1.12 (±2.876)	1.16 (±0.189)
1 x 10^{10}	1.12 (±0.182)	0.99 (±0.159)
Control (0 CFU/mL)	0.87 (±0.114)	0.86 (±0.128)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.85 Feed conversion ratio (FCR) at day 21 for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	1.27 (±0.268)	1.20 (±0.405)
1 x 10^7	1.78 (±6.399)[1]	1.03 (±0.413)[2]
1 x 10^8	1.50 (±0.409)[1]	1.05 (±0.041)[2]
1 x 10^9	1.45 (±0.971)	1.12 (±2.876)
1 x 10^{10}	1.28 (±0.325)	1.12 (±0.182)
Control (0 CFU/mL)	0.87 (±0.114)	0.87 (±0.114)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.86 Feed conversion ratio (FCR) at day 21 for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	1.18 (±0.598)	1.04 (±0.351)
1 x 10^7	1.24 (±0.517)	1.09 (±0.543)
1 x 10^8	1.54 (±4.056)	1.07 (±0.064)
1 x 10^9	1.74 (±0.658)[1]	1.16 (±0.189)[2]
1 x 10^{10}	1.20 (±0.509)	0.99 (±0.159)
Control (0 CFU/mL)	0.86 (±0.128)	0.86 (±0.128)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.87 Cumulative feed conversion ratio (FCR) for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	1.27 (±0.117)^{a}	1.18 (±0.146)^{ac}
1 x 10^7	1.80 (±0.250)^{b1}	1.24 (±0.203)^{a2}
1 x 10^8	1.50 (±0.100)^{abc}	1.54 (±0.340)^{a}
1 x 10^9	1.45 (±0.112)^{ab1}	1.74 (±0.150)^{b2}
1 x 10^{10}	1.30 (±0.110)^{a}	1.20 (±0.182)^{ac}
Control (0 CFU/mL)	0.87 (±0.012)^{c}	0.86 (±0.054)^{c}

^{abc}Column means with same superscript do not differ significantly (P>0.0033)

^{12}Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.88 Cumulative feed conversion ratio (FCR) for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	1.20 (±0.193)	1.04 (±0.030)
1 x 10^7	1.03 (±0.102)	1.09 (±0.169)
1 x 10^8	1.05 (±0.054)	1.07 (±0.003)
1 x 10^9	1.12 (±0.130)	1.20 (±0.030)
1 x 10^{10}	1.12 (±0.050)	0.99 (±0.061)
Control (0 CFU/mL)	0.87 (±0.012)	0.86 (±0.054)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.89 Cumulative feed conversion ratio (FCR) for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	1.27 (±0.117)	1.20 (±0.193)
1 x 10^7	1.80 (±0.250)[1]	1.03 (±0.102)[2]
1 x 10^8	1.50 (±0.100)[1]	1.05 (±0.054)[2]
1 x 10^9	1.45 (±0.112)[1]	1.12 (±0.130)[2]
1 x 10^{10}	1.30 (±0.110)	1.12 (±0.050)
Control (0 CFU/mL)	0.87 (±0.012)	0.87 (±0.012)

[12]Row means with same superscript do not differ significantly (P>0.05)

*Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.90 Cumulative feed conversion ratio (FCR) for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	1.18 (±0.146)	1.04 (±0.030)
1 x 10^7	1.24 (±0.203)	1.09 (±0.169)
1 x 10^8	1.54 (±0.340)	1.07 (±0.003)
1 x 10^9	1.74 (±0.150)	1.20 (±0.030)
1 x 10^{10}	1.20 (±0.182)	0.99 (±0.061)
Control (0 CFU/mL)	0.86 (±0.054)	0.86 (±0.054)

* Cyclophosphamide given at 3 mg/mL via oral gavage

There were no mortalities in the control group of birds. There did not appear to be any ST dose response regarding mortalities. Birds challenged with the Salmonella on day 7 had the highest number of mortalities (27.5% of the birds), while those challenged on day 4 had a mortality rate of 12.5%. The highest number of mortalities for the birds challenged on day 7 occurred in the groups that received CY, with the highest mortalities occurring in week 2 of the trial. The highest number of deaths for those birds challenged on day 4 of the trial occurred in week 1.

3.2.3) Serum biochemical profile

Exposure to Salmonella appeared to decrease albumin levels (Table 3.93 and Table 3.94), increase globulin levels (Table 3.97 and Table 3.98) and increase TSP levels (Table 3.105 and Table 3.106). Salmonella challenged birds had increased AST levels except for the

groups challenged on day 7 that received CY, where it appeared to decrease the AST levels (Table 3.109 and Table 3.110).

Birds challenged with Salmonella on day 4 had higher albumin levels (Table 3.91 and Table 3.92), higher globulin levels (Table 3.95 and Table 3.96), higher TSP levels (Table 3.103 and Table 3.104) and higher AST levels (Table 3.107 and Table 3.108) than the birds which were challenged on day 7 of the trial. Exposure to CY had no significant effect on serum levels (Table 3.93, Table 3.94, Table 3.97, Table 3.98, Table 3.105, Table 3.106, Table 3.109 and Table 3.110).

Table 3.91 Albumin levels for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	14.80 (±0.283)	13.90 (±0.361)
1 x 10^7	15.77 (±1.301)	15.30 (±0.424)
1 x 10^8	14.10 (±1.473)	15.37 (±0.351)
1 x 10^9	15.33 (±0.702)[1]	13.53 (±1.069)[2]
1 x 10^{10}	13.83 (±1.069)	13.60 (±0.866)

[12]Row means with the same superscript do not differ significantly (P > 0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.92 Albumin levels for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	12.50 (±2.121)[1]	14.13 (±0.380)[2]
1 x 10^7	14.43 (±1.069)	14.03 (±0.590)
1 x 10^8	13.47 (±0.603)	14.80 (±0.436)
1 x 10^9	14.35 (±0.071)	13.67 (±0.902)
1 x 10^{10}	13.95 (±0.071)	13.97 (±0.252)

[12]Row means with the same superscript do not differ significantly (P > 0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.93 Albumin levels for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY^-	CY^+
1 x 10^6	14.80 (±0.283)[1]	12.50 (±2.121)[a2]
1 x 10^7	15.77 (±1.301)	14.43 (±1.069)[ab]
1 x 10^8	14.10 (±1.473)	13.47 (±0.603)[a]
1 x 10^9	15.33 (±0.702)	14.35 (±0.071)[ab]
1 x 10^{10}	13.83 (±1.069)	13.95 (±0.071)[ab]
Control (0 CFU/mL)	14.17 (±0.306)[1]	15.63 (±0.451)[b2]

[ab]Column means with the same superscript do not differ significantly (P > 0.0033)

[12]Row means with the same subscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.94 Albumin levels for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY^-	CY^+
1 x 10^6	13.90 (±0.361)	14.13 (±0.380)
1 x 10^7	15.30 (±0.424)	14.03 (±0.590)
1 x 10^8	15.37 (±0.351)	14.80 (±0.436)
1 x 10^9	13.53 (±1.069)	13.67 (±0.902)
1 x 10^{10}	13.60 (±0.866)	13.97 (±0.252)
Control (0 CFU/mL)	14.17 (±0.306)[1]	15.63 (±0.451)[2]

[12]Row means with the same subscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.95 Globulin levels for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	14.85 (±1.768)	12.63 (±3.231)
1 x 10^7	14.33 (±1.518)	12.55 (±0.354)
1 x 10^8	15.50 (±2.553)[1]	12.13 (±1.168)[2]
1 x 10^9	14.10 (±1.212)	12.03 (±1.365)
1 x 10^{10}	13.07 (±1.617)	12.10 (±1.153)

[12]Row means with the same superscript do not differ significantly (P > 0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.96 Globulin levels for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	14.10 (±1.980)	11.47 (±2.043)
1 x 10^7	12.97 (±3.190)	11.77 (±1.656)
1 x 10^8	12.60 (±2.140)	13.30 (±0.473)
1 x 10^9	12.75 (±2.616)	12.00 (±0.300)
1 x 10^{10}	11.45 (±1.485)	12.87 (±1.903)

*Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.97 Globulin levels for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY^-	CY^+
1 x 10^6	14.85 (±1.768)	14.10 (±1.980)
1 x 10^7	14.33 (±1.518)	12.97 (±3.190)
1 x 10^8	15.50 (±2.553)	12.60 (±2.140)
1 x 10^9	14.10 (±1.212)	12.75 (±2.616)
1 x 10^{10}	13.07 (±1.617)	11.45 (±1.485)
Control (0 CFU/mL)	10.90 (±1.609)	12.00 (±1.212)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.98 Globulin levels for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY^-	CY^+
1 x 10^6	12.63 (±3.231)	11.47 (±2.043)
1 x 10^7	12.55 (±0.354)	11.77 (±1.656)
1 x 10^8	12.13 (±1.168)	13.30 (±0.473)
1 x 10^9	12.03 (±1.365)	12.00 (±0.300)
1 x 10^{10}	12.10 (±1.153)	12.87 (±1.903)
Control (0 CFU/mL)	10.90 (±1.609)	12.00 (±1.212)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.99 Albumin:globulin ratio levels for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	1.00 (±0.099)	1.15 (±0.288)
1 x 10^7	1.11 (±0.168)	1.22 (±0.071)
1 x 10^8	0.93 (±0.160)[1]	1.27 (±0.111)[2]
1 x 10^9	1.09 (±0.136)	1.14 (±0.155)
1 x 10^{10}	1.07 (±0.173)	1.14 (±0.172)

[12]Row means with the same superscript do not differ significantly (P > 0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.100 Albumin:globulin ratio levels for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.91 (±0.276)[1]	1.26 (±0.202)[2]
1 x 10^7	1.15 (±0.210)	1.20 (±0.121)
1 x 10^8	1.09 (±0.210)	1.12 (±0.046)
1 x 10^9	1.15 (±0.226)	1.14 (±0.095)
1 x 10^{10}	1.23 (±0.170)	1.10 (±0.180)

[12]Row means with the same superscript do not differ significantly (P > 0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.101 Albumin:globulin ratio levels for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY^-	CY^+
1 x 10^6	1.00 (±0.099)	0.91 (±0.276)
1 x 10^7	1.11 (±0.168)	1.15 (±0.210)
1 x 10^8	0.93 (±0.160)	1.09 (±0.210)
1 x 10^9	1.09 (±0.136)	1.15 (±0.226)
1 x 10^{10}	1.07 (±0.173)	1.23 (±0.170)
Control (0 CFU/mL)	1.32 (±0.184)	1.31 (±0.157)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.102 Albumin:globulin ratio levels for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY^-	CY^+
1 x 10^6	1.15 (±0.288)	1.26 (±0.202)
1 x 10^7	1.22 (±0.071)	1.20 (±0.121)
1 x 10^8	1.27 (±0.111)	1.12 (±0.046)
1 x 10^9	1.14 (±0.155)	1.14 (±0.095)
1 x 10^{10}	1.14 (±0.172)	1.10 (±0.180)
Control (0 CFU/mL)	1.32 (±0.184)	1.31 (±0.157)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.103 Total serum protein (TSP) levels for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	29.65 (±2.051)	26.53 (±2.873)
1 x 10^7	30.10 (±1.652)	27.85 (±0.071)
1 x 10^8	29.60 (±3.160)	27.50 (±1.400)
1 x 10^9	29.43 (±1.012)[1]	25.60 (±1.665)[2]
1 x 10^{10}	26.90 (±1.473)	25.70 (±0.361)

[12]Row means with the same superscript do not differ significantly (P > 0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.104 Total serum protein (TSP) levels for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	26.60 (±0.141)	25.60 (±2.170)
1 x 10^7	27.40 (±4.051)	25.80 (±2.227)
1 x 10^8	26.03 (±2.303)	28.10 (±0.702)
1 x 10^9	27.10 (±2.687)	25.70 (±0.780)
1 x 10^{10}	25.40 (±1.414)	26.83 (±1.804)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.105 Total serum protein (TSP) levels for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY^-	CY^+
1 x 10^6	29.65 (±2.051)^{ab}	26.60 (±0.141)
1 x 10^7	30.10 (±1.652)^{a}	27.40 (±4.051)
1 x 10^8	29.60 (±3.160)^{ab1}	26.03 (±2.303)^{2}
1 x 10^9	29.43 (±1.012)^{ab}	27.10 (±2.687)
1 x 10^{10}	26.90 (±1.473)^{ab}	25.40 (±1.414)
Control (0 CFU/mL)	25.10 (±1.890)^{b}	27.63 (±0.874)

^{ab}Column means with the same superscript do not differ significantly (P > 0.0033)

^{12}Row means with the same subscript do not differ significantly (P> 0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.106 Total serum protein (TSP) levels for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY^-	CY^+
1 x 10^6	26.53 (±2.873)	25.60 (±2.170)
1 x 10^7	27.85 (±0.071)	25.80 (±2.227)
1 x 10^8	27.50 (±1.400)	28.10 (±0.702)
1 x 10^9	25.60 (±1.665)	25.70 (±0.780)
1 x 10^{10}	25.70 (±0.361)	26.83 (±1.804)
Control (0 CFU/mL)	25.10 (±1.890)	27.63 (±0.874)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.107 Aspartate transaminase (AST) levels for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	719.0 (±28.28)	368.0 (±257.5)
1 x 10^7	1083 (±490.9)[1]	207.5 (±16.26)[2]
1 x 10^8	325.0 (±129.5)	214.3 (±14.57)
1 x 10^9	873.7 (±934.5)[1]	226.3 (±19.30)[2]
1 x 10^{10}	262.7 (±24.44)	212.7 (±2.082)

[12]Row means with the same superscript do not differ significantly (P > 0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.108 Aspartate transaminase (AST) levels for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	212.0 (±7.071)	247.3 (±22.68)
1 x 10^7	493.7 (±224.9)	217.7 (±10.02)
1 x 10^8	262.3 (±111.9)	256.3 (±41.62)
1 x 10^9	532.0 (±405.9)	211.7 (±19.50)
1 x 10^{10}	603.0 (±534.6)	256.7 (±90.53)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.109 Aspartate transaminase (AST) levels for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY^-	CY^+
1 x 10^6	719.0 (±28.28)^{ab}	212.0 (±7.071)
1 x 10^7	1083 (±490.9)^{a1}	493.7 (±224.9)^{2}
1 x 10^8	325.0 (±129.5)^{b}	262.3 (±111.9)
1 x 10^9	873.7 (±934.5)^{ab}	532.0 (±405.9)
1 x 10^{10}	262.7 (±24.44)^{b}	603.0 (±534.6)
Control (0 CFU/mL)	220.3 (±45.52)^{b}	280.7 (±30.86)

^{ab}Column means with the same superscript do not differ significantly (P > 0.0033)

^{12}Row means with the same subscript do not differ significantly (P> 0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.110 Aspartate transaminase (AST) levels for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY^-	CY^+
1 x 10^6	368.0 (±257.5)	247.3 (±22.68)
1 x 10^7	207.5 (±16.26)	217.7 (±10.02)
1 x 10^8	214.3 (±14.57)	256.3 (±41.62)
1 x 10^9	226.3 (±19.30)	211.7 (±19.50)
1 x 10^{10}	212.7 (±2.08)	256.7 (±90.53)
Control (0 CFU/mL)	220.3 (±45.52)	280.7 (±30.86)

* Cyclophosphamide given at 3 mg/mL via oral gavage

3.2.4) Intestinal damage

No lesions were found in any of the control groups of birds. Chi-Square analysis was done for the number of broilers presented with lesions (Table 3.111, Table 3.112 and Table 3.113). Using a significance level of 0.05 it was found that there were no statistically significant differences between the treatment levels and groups for the number of chicks in which lesions occurred. However, it would appear that there were fewer birds with lesions in the groups exposed to Salmonella and CY.

Table 3.111 The number of chicks which had gastrointestinal lesions according to the different levels of *Salmonella typhimurium* (ST) challenge and exposure to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-)

ST level (CFU/mL)	CY+	CY-
1 x 10^6	6	7
1 x 10^7	16	12
1 x 10^8	7	11
1 x 10^9	11	13
1 x 10^{10}	6	13

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.112 The number of chicks which had gastrointestinal lesions according to infection day and exposure to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-)

Day	CY+	CY-
4	20	32
7	26	24

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.113 The number of chicks which had gastrointestinal lesions according to the different levels of *Salmonella typhimurium* (ST) challenge and infection day

ST level (CFU/mL)	Day 4	Day 7
1 x 10^6	6	7
1 x 10^7	16	12
1 x 10^8	8	9
1 x 10^9	13	11
1 x 10^{10}	9	10

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.114, Table 3.115 Table 3.116 and Table 3.117 were based on the pen averages for the severity of lesions on a scale of 0 = no lesions, 1 = mild lesions, 2 = moderate lesions and 3 = severe lesions.

The lesion data results shown in Table 3.114 and Table 3.115 showed that CY appeared to have no influence on the severity of the lesions found. Similarly, Table 3.116 and Table 3.117 showed that the age when the chicks were challenged with Salmonella had no effect on the severity of the intestinal lesions.

Table 3.114 Gastrointestinal lesion severity for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	0.11 (±0.192)[a]	0.33 (±0.334)
1 x 10^7	0.33 (±0)[a]	0.56 (0.193)
1 x 10^8	0.33 (±0)[a]	0.33 (±0.334)
1 x 10^9	1.22 (±0.694)[b1]	0.56 (±0.509)[2]
1 x 10^{10}	0.50 (±0.167)[ab]	0.33 (±0.578)
Control (0 CFU/mL)	0 (±0)[a]	0 (±0)

[ab]Column means with same superscript do not differ significantly (P>0.0033)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.115 Gastrointestinal lesion severity for broilers exposed to cyclophosphamide (CY+)* and those not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 7 days of age (value ± standard deviation of the mean)

ST level (CFU/mL)	CY-	CY+
1 x 10^6	0.22 (±0.192)	0.33 (±0.334)
1 x 10^7	0.33 (±0.334)	0.61 (±0.347)
1 x 10^8	0.33 (±0.334)	0.67 (±0.334)
1 x 10^9	0.56 (±0.193)	0.42 (±0.221)
1 x 10^{10}	0.44 (±0.509)	0.56 (±0.193)
Control (0 CFU/mL)	0 (±0)	0 (±0)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.116 Gastrointestinal lesion severity for broilers not exposed to cyclophosphamide (CY-), and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.11 (±0.192)	0.22 (±0.192)
1 x 10^7	0.33 (±0)	0.33 (±0.334)
1 x 10^8	0.33 (±0)	0.33 (±0.334)
1 x 10^9	1.22 (±0.694)[1]	0.56 (±0.193)[2]
1 x 10^{10}	0.50 (±0.167)	0.44 (±0.509)

[12]Row means with same superscript do not differ significantly (P>0.05)

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.117 Gastrointestinal lesion severity for broilers not exposed to cyclophosphamide (CY+)*, and challenged with different levels of *Salmonella typhimurium* (ST) at 4 and 7 days of age (value ± standard deviation of the mean)

	Day of challenge	
ST level (CFU/mL)	**4**	**7**
1 x 10^6	0.33 (±0.334)	0.33 (±0.334)
1 x 10^7	0.56 (0.193)	0.61 (±0.347)
1 x 10^8	0.33 (±0.334)	0.67 (±0.334)
1 x 10^9	0.56 (±0.509)	0.42 (±0.221)
1 x 10^{10}	0.33 (±0.578)	0.56 (±0.193)

* Cyclophosphamide given at 3 mg/mL via oral gavage

3.2.5) Histopathology

Histopathology results for the control groups predominantly showed signs of "nothing abnormal or nothing remarkable" (Table 3.118 – 3.125). There did not appear to be any significant differences of CY exposure or day of Salmonella challenge on the organs. The control groups did display some signs of bursal atrophy, mild lymphocyte loss, mild caecal diffuse and multifocal typhilitis, mild hepatic necrosis as well as vacuolar change in the liver.

No conclusive evidence was provided for the effects of Salmonella and Cyclophosphamide on the sampled organs. The Salmonella challenged groups showed signs of "nothing abnormal or nothing remarkable" predominantly in the caecal, spleen and liver samples. Exposure to CY and the date of Salmonella challenge did not appear to display conclusive differences.

In the bursa of Salmonella challenged birds there were signs of marked atrophy and mild to moderate lymphocyte loss. The caeca displayed signs of mild diffuse- and mild multifocal typhilitis, and in one instance mild congestion. The SS-sheaths were prominent in many of the spleen samples, especially for the birds challenged with Salmonella on day 4. Liver samples showed signs of vacuolar change, hepatic necrosis, hepatitis and mild perivascular granulopoeisis.

3.3) Discussion

Cloacal swabs taken from the non-challenged birds that received cyclophosphamide and from the non-challenged birds that did not receive cyclophosphamide tested negative for Salmonella, while the swabs from the challenged birds that received cyclophosphamide and from the challenged birds that did not receive cyclophosphamide tested positive for Salmonella. The biosecurity measures employed were thus effective in keeping the non-challenged birds free from Salmonella, and the use of cyclophosphamide did not inhibit Salmonella colonisation in the challenged birds.

Table 3.118 The number of birds showing histopathology results for the bursa of chicks injected with Cyclophosphamide (CY) at 4 days of age and challenged with different levels of *Salmonella typhimurium* (ST)

Effect seen	O CFU Salmonella		1×10^6 CFU Salmonella		1×10^7 CFU Salmonella		1×10^8 CFU Salmonella		1×10^9 CFU Salmonella		1×10^{10} CFU Salmonella	
	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-
NA/NR	6	2	1	0	0	1	2	0	0	1	0	1
Moderate atrophy	6	2	0	0	0	1	0	0	0	1	0	0
Marked atrophy	0	2	2	3	3	1	2	2	0	3	4	0
Mild lymphocyte loss	0	0	1	1	1	3	2	4	4	1	2	3
Moderate lymphocyte loss	0	0	0	2	1	0	0	0	0	0	0	0

NA/NR = Nothing abnormal/ nothing remarkable

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.119 The number of birds showing histopathology results for the bursa of chicks injected with Cyclophosphamide (CY) at 7 days of age and challenged with different levels of *Salmonella typhimurium* (ST)

Effect seen	O CFU Salmonella		1 x 10^6 CFU Salmonella		1 x 10^7 CFU Salmonella		1 x 10^8 CFU Salmonella		1 x 10^9 CFU Salmonella		1 x 10^{10} CFU Salmonella	
	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-
NA/NR	6	2	1	1	3	4	0	2	2	0	1	2
Moderate atrophy	6	2	0	0	1	1	1	0	1	1	0	0
Marked atrophy	0	2	3	0	1	1	3	1	1	3	4	1
Mild lymphocyte loss	0	0	1	3	1	0	1	3	2	0	1	3
Severe atrophy	0	0	1	1	0	0	0	0	0	0	0	0
Mild atrophy	0	0	0	1	0	0	1	0	0	0	0	0

NA/NR = Nothing abnormal/ nothing remarkable

* Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.120 The number of chicks showing histopathology results for the caeca of chicks injected with CY at 4 days of age and challenged with different levels of *Salmonella typhimurium* (ST)

Effect seen	O CFU Salmonella		1×10^6 CFU Salmonella		1×10^7 CFU Salmonella		1×10^8 CFU Salmonella		1×10^9 CFU Salmonella		1×10^{10} CFU Salmonella	
	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-
NA/NR	12	8	3	3	2	2	2	0	0	0	2	2
Mild diffuse typhilitis	0	3	0	3	4	3	4	5	4	3	3	1
Mild multifocal typhilitis	0	1	1	0	0	2	0	1	0	3	1	1

NA/NR = Nothing abnormal/ nothing remarkable

*Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.121 The number of birds showing histopathology results for the caeca of chicks injected with CY at 7 days of age and challenged with different levels of *Salmonella typhimurium* (ST)

Effect seen	O CFU Salmonella		1×10^6 CFU Salmonella		1×10^7 CFU Salmonella		1×10^8 CFU Salmonella		1×10^9 CFU Salmonella		1×10^{10} CFU Salmonella	
	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-
NA/NR	12	8	3	2	5	5	6	6	2	0	1	1
Mild diffuse typhilitis	0	3	0	0	0	1	0	0	2	2	5	4
Mild multifocal typhilitis	0	1	3	4	0	0	0	0	2	2	0	0
Mild congestion	0	0	0	0	1	0	0	0	0	0	0	0
Mild multifocal acute typhilitis	0	0	0	0	0	0	0	0	0	0	0	1

NA/NR = Nothing abnormal/ nothing remarkable

*Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.122 The number of birds showing histopathology results for the spleen of chicks injected with CY at 4 days of age and challenged with different levels of *Salmonella typhimurium* (ST)

Effect seen	O CFU Salmonella		1×10^6 CFU Salmonella		1×10^7 CFU Salmonella		1×10^8 CFU Salmonella		1×10^9 CFU Salmonella		1×10^{10} CFU Salmonella	
	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-
NA/NR	12	12	4	5	2	5	4	3	0	2	3	2
SS-sheaths prominent	0	0	0	1	3	1	2	3	4	4	3	2

NA/NR = Nothing abnormal/ nothing remarkable

*Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.123 The number of birds showing histopathology results for the spleen of chicks injected with CY at 7 days of age and challenged with different levels of *Salmonella typhimurium* (ST)

Effect seen	O CFU Salmonella		1 x 10^6 CFU Salmonella		1 x 10^7 CFU Salmonella		1 x 10^8 CFU Salmonella		1 x 10^9 CFU Salmonella		1 x 10^{10} CFU Salmonella	
	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-
NA/NR	12	12	6	4	5	5	5	5	5	2	5	5
SS-sheaths prominent	0	0	0	2	1	1	1	1	1	2	1	1

NA/NR = Nothing abnormal/ nothing remarkable

*Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.124 The number of chicks showing histopathology results for the liver of chicks injected with CY at 4 days of age and challenged with different levels of *Salmonella typhimurium* (ST)

Effect seen	O CFU Salmonella		1 x 10^6 CFU Salmonella		1 x 10^7 CFU Salmonella		1 x 10^8 CFU Salmonella		1 x 10^9 CFU Salmonella		1 x 10^{10} CFU Salmonella	
	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-
NA/NR	12	11	2	2	1	1	4	2	2	5	5	4
Mild fatty change	0	1	0	0	1	1	0	0	0	0	0	0
Moderate vacuolar change	0	0	1	0	1	1	0	0	1	0	0	0
Mild vacuolar change	0	0	0	2	0	0	0	1	1	0	1	0
Marked vacuolar change	0	0	0	0	1	0	0	0	0	0	0	0
Mild multifocal acute hepatic necrosis and hepatitis	0	0	1	0	0	0	0	0	0	0	0	0
Moderate chronic active hepatitis and perivascular granulopoeisis	0	0	0	1	0	0	0	0	0	0	0	0
Moderate multifocal to coalescing hepatic necrosis/hepatitis	0	0	0	1	0	0	0	0	0	0	0	0
Moderate multifocal to coalescing hepatic necrosis/hepatitis and perivascular granulopoeisis	0	0	0	0	1	0	0	0	0	0	0	0
Mild multifocal hepatic necrosis and perivascular granulopoeisis	0	0	0	0	0	1	2	0	0	0	0	0
Mild perivascular granulopoeisis	0	0	0	0	0	0	0	1	0	1	0	0
Moderate multifocal acute hepatic necrosis/hepatitis	0	0	0	0	0	1	0	0	0	0	0	0
Mild multifocal chronic active granulomatous hepatitis	0	0	0	0	0	1	0	0	0	0	0	0

NA/NR = Nothing abnormal/ nothing remarkable

*Cyclophosphamide given at 3 mg/mL via oral gavage

Table 3.125 The number of birds showing histopathology results for the liver of chicks injected with CY at 7 days of age and challenged with different levels of *Salmonella typhimurium* (ST)

Effect seen	O CFU Salmonella		1×10^6 CFU Salmonella		1×10^7 CFU Salmonella		1×10^8 CFU Salmonella		1×10^9 CFU Salmonella		1×10^{10} CFU Salmonella	
	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-	CY+	CY-
NA/NR	12	11	1	1	3	2	0	3	5	1	6	5
Mild fatty change	0	1	0	0	0	0	0	0	1	0	0	1
Moderate vacuolar change	0	0	1	3	0	1	1	2	0	0	0	0
Moderate vacuolar degeneration	0	0	0	1	0	0	0	0	0	0	0	0
Mild vacuolar change	0	0	5	1	2	1	3	1	0	2	0	0
Severe chronic active multifocal to bridging granulomatous hepatitis	0	0	0	0	1	0	0	0	0	0	0	0
Marked multifocal to coalescing hepatic necrosis and heterophils infiltrate	0	0	0	0	0	1	0	0	0	0	0	0
Marked vacuolar change	0	0	0	0	0	0	1	0	0	0	0	0
Scattered small lymphoid aggregates	0	0	0	0	0	0	1	0	0	0	0	0
Moderate hepatic necrosis and perivascular granulopoeisis	0	0	0	0	0	0	0	0	0	1	0	0

NA/NR = Nothing abnormal/ nothing remarkable

*Cyclophosphamide given at 3 mg/mL via oral gavage

3.3.1) Organ weights

According to Calnek *et al.* (1991), ST infection results in enlarged liver, spleen and kidneys. This was confirmed by the trial results where all of the organ weights, except for the heart, increased when the birds were exposed to Salmonella. Early challenge with Salmonella (day 4) resulted in higher organ weights than those birds that were challenged later (day 7), although no effect was seen on the heart or the liver. Cyclophosphamide did not have any significant effect on organ weight.

3.3.2) Broiler performance

According to a trial conducted by Du & Wang (2005) there was a reduction in body weight gain for chicks infected with high doses of ST, but that there were no significant differences for the body weights between the groups. Salmonella challenged birds had lower BW, ADG and FI and higher FCR than the non-challenged birds. Early challenge with Salmonella resulted in higher BW and ADG and lower FI and FCR in the first week of the trial when compared to birds that were challenged later. During week 2 of the trial BW, FI, ADG and FCR decreased in the early challenged birds exposed to cyclophosphamide. In the final week of the trial early challenge with Salmonella resulted in lower BW and ADG with higher FI and FCR than the birds challenged later in the trial.

Immunosuppressed flocks may have increased incidence of secondary infections and poor feed conversion. Cyclophosphamide had no effect on broiler performance (Corrier *et al*., 1991; El-Abasy *et al*., 2004; He *et al*., 2007; Reynolds & Maraqa, 1999). Higher mortality rates were found for those birds treated with CY (Corrier *et al*., 1991; El-Abasy *et al*., 2004; He *et al*., 2007; Reynolds & Maraqa, 1999).

3.3.3) Serum biochemical profile

According to Duke (1993), plasma makes up between 55-70% of the blood, and plasma protein synthesis decreases in severe liver damage. Plasma proteins consist of two major types: albumin and globulin. Plasma protein synthesis decreases with severe liver damage (Tung *et al*., 1975). Albumin is the most abundant protein in the plasma, and is the major protein produced by the liver (Duke, 1993; Frandson & Spurgeon, 1992). Albumin is important in the binding and transporting of many substances in the blood and is responsible for about 80% of the total potential osmotic pressure (oncotic pressure) of the plasma as it is a high molecular weight protein that does not pass readily through the vessel or capillary walls it therefore aids in keeping fluid in the vascular system (Frandson & Spurgeon, 1992). Chickens treated with CY had significantly lower serum antibody production (Glick, 1986; He *et al*., 2007; Kim *et al*., 2003; Reynolds & Maraqa, 1999).

Globulin is a reactive protein and a plasma precursor with Gamma-globulins being stimulated by the presence of antigens and synthesised by the plasma cells (Frandson & Spurgeon, 1992) and lymphocytes containing the antibodies known as immunoglobulins (Duke, 1993). Gamma-globulin is associated with immunity and resistance to disease. The Gamma-globulin content of the blood therefore increases following vaccination and during recovery from disease. Beta-globulin transferin combines with and carries iron from the blood capillaries in the mucosa. The Alpha- and Beta-

globulins are synthesised in the liver. Globulin and albumin are simple proteins that yield only amino acids or their derivatives upon hydrolysis (Duke, 1993).

In this trial, birds challenged with Salmonella had lower albumin levels and higher globulin, TSP and AST levels than the non-challenged birds. Early challenge with Salmonella resulted in higher serum levels than in the later challenged birds.

Chickens treated with CY had significantly lower serum antibody production (Glick, 1986; He *et al*., 2007; Kim *et al*., 2003; Reynolds & Maraqa, 1999). This was also found to be the case in this trial where the birds that were challenged with Salmonella and exposed to Cyclophosphamide had lower serum levels than in the birds not exposed to Cyclophosphamide. Cyclophosphamide had no effect on the non-challenged birds.

Total plasma proteins are a common endpoint utilised to estimate avian body condition (Rajman *et al*., 2006). Birds infected with ST showed significantly elevated antibody levels in the serum than those of the control birds (Beal *et al*., 2004; Beal *et al*., 2006; Du & Wang, 2005; Lee *et al*., 1983; Okamura *et al*., 2007) rising to a peak at day 29 of infection (Lee *et al*., 1983).

Aspartate is catabolised to produce fumarate by way of the urea cycle. Birds excrete excess amino nitrogen as uric acid (Duke, 1993). Serum prepared from blood sampled taken at the slaughter of the animals is used to determine the activity of aspartate aminotransferase (AST), and is used as a marker of liver injury. Alteration in the activity of AST has been used to assess liver toxicity produced by chemicals (Adav & Govindwar, 1997). Plasma AST activity may reflect changes in hepatic function and can be used as a biochemical indicator for hepatic damage and as a marker enzyme for hepatocellular necrosis (Bintvihok & Kositcharoenkul, 2006; Coulombe *et al*., 2005; Frankič *et al*., 2006; Han *et al*., 2008; Rishi *et al*., 2006).

According to Reddy *et al.* (2006) AST levels increase in cases of hepatocellular and muscular damage making it a good tool for assessing liver and muscle damage. Corduk *et al.* (2007) confirmed this by stating that an increase in AST activity is an indicator of a progressive liver cell injury followed by an increased production of reactive oxygen species (ROS) due to external factors such as heat, trauma, infection, toxin and exercises.

According to Brenes *et al.* (2003), Corduk (2007) and Rajman *et al.* (2006) AST may reflect change in hepatic function in addition to alterations in muscle membrane permeability. Plasma AST is not so specific and sensitive to hepatocellular damage in birds as it is in mammals (Brenes *et al*., 2003; Rajman *et al*., 2006), although according to Denli *et al*. (2004) the activities of AST in serum is a sensitive indicator of acute hepatic necrosis.

3.3.4) Intestinal damage: lesions

According to Berndt & Methner (2001, 2004), there were no signs of intestinal inflammation in birds that were orally administered ST vaccine although some birds challenged with ST showed a slight inflammation of the intestine in the first week after infection. In trials done on mice infected with ST results included atrophy with ischemic necrosis in the small intestine mucous layers (Lee *et al*., 2006; Rishi *et al*., 2006).

Post mortem, findings can range from complete absence of visible lesions to a septicaemic carcass. Some birds may show signs of lesions identical to any acute septicaemia. Lesions may be absent in extremely severe outbreaks. Adult birds can show signs of necrotic ulcers in the intestines, although chronically infected adults frequently exhibit no lesions (Jordan & Pattison, 1996).

No lesions were found in any of the control groups of birds. It would appear that there were fewer birds with lesions in the groups exposed to Salmonella and CY. Cyclophosphamide appeared to have no influence on the severity of the lesions found, while the day of Salmonella challenge too had no effect on the severity of the intestinal lesions.

3.3.5) Histopathological results

Allameh *et al*. (2005), Coulombe *et al*. (2005), Denli & Okan (2006) and Méndez-Albores *et al*. (2007) found extensive liver damage in trials conducted on chickens, with hepatocellular necrosis, multifocal fatty degeneration with large fat droplets displacing the nucleus resulting in fatty liver. There was leukocyte infiltration, congestion, hyperplasia of the epithelium as well as biliary hyperplasia. The livers appeared friable and yellowish in colouration. According to Calnek *et al.* (1991), young birds showed signs of congested liver and spleen with haemorrhagic streaks or necrotic foci, congested kidneys as well as pericarditis with adhesions, while adult birds showed signs of congested and swollen livers, spleen and kidneys with haemorrhagic or necrotic enteritis, pericarditis and peritonitis. This is supported by Jordan & Pattison (1996), who found signs of swollen lungs, liver, spleen and kidneys. Unabsorbed yolk sacs have been seen in young chicks. Necrotic lesions in the lungs, liver and heart, peritonitis, typhlitis and haemorrhagic enteritis are all symptoms that can be seen with Salmonella infection.

In trials done on mice infected with ST results included atrophy with ischemic necrosis in the small intestine mucous layers as well as severe haemorrhagic necrosis within the pulp of the spleen. The liver was congested with polymorphonuclear leukocyte infiltration, fatty deposits, structural disintegration of hepatic plates, haemorrhage, necrosis and the presence of necrotic foci (Lee *et al*., 2006; Rishi *et al*., 2006).

No conclusive evidence was provided for the effects of Salmonella and Cyclophosphamide on the sampled organs. The Salmonella challenged groups showed signs of “nothing abnormal or nothing

remarkable" predominantly in the caecal, spleen and liver samples. Exposure to CY and the date of Salmonella challenge did not appear to display conclusive differences.

In the bursa of Salmonella challenged birds there were signs of marked atrophy and mild to moderate lymphocyte loss. The caeca displayed signs of mild diffuse- and mild multifocal typhilitis, and in one instance mild congestion. The SS-sheaths were prominent in many of the spleen samples, especially for the birds challenged with Salmonella on day 4. Liver samples showed signs of vacuolar change, hepatic necrosis, hepatitis and mild perivascular granulopoeisis.

3.4) Conclusion

According to the results obtained from broiler performance, organ weights, lesions in the GIT and the serum biochemical profile, the best age for challenging birds with ST would be day 4 after hatch. This pilot trial also indicated that it will not be necessary to treat birds with CY prior to ST challenge.

The application of the CY is time and labour intensive as it needs to be done every day for the first four days of the chicks life, and as there were no real significant differences between the CY levels the use of CY as an immunosuppressive agent is not required. This is supported by the fact that the cloacal swabs taken from the CY- birds that were challenged with ST were positive for the presence of Salmonella, and so it can be assumed that the strain of Salmonella typhimurium used is virulent enough to infect the chicks. Mortalities were highest for day 7 challenge.

From the results obtained, it was decided that no CY would be used in the next trial and that the chicks would be challenged with ST on day 4 of the trial. It was also decided that two ST levels would be used, namely 1 x 10^5 CFU/mL and 1 x 10^{10} CFU/mL along with a control group. Broiler performance and serum biochemical profile would be done as in this trial, while organ weights would only be taken for the liver, duodenum, jejenum, ileum and caeca. For the histopathology, it was decided that only the liver, caeca and bursa would be analysed.

Chapter 4

Salmonella typhimurium infection in boilers and its effects on gastrointestinal health and function

Although *Salmonella typhimurium* (ST) mainly causes food poisoning in humans, infections may progress to bacteraemia, particularly in immune-compromised people, such as AIDS patients (Cardinale *et al.,* 2004). ST is also the most common serovar causing cardiovascular, bone and joint infections (Lan *et al.,* 2007). Salmonella infection not only causes salmonellosis in humans, but also can result in great economic losses in the typically narrow-margin, high-volume broiler business due to reduced growth rates and mortalities. Setbacks in the prevention and confinement of this disease result from the fact that chickens can be asymptomatic carriers of Salmonella (Cox, 1995; Fernández *et al.,* 2001; Amy *et al*., 2004; Bohez *et al*., 2006).

Over the last decade, the use of antibiotics and attenuated vaccines to restrain or prevent Salmonella infection in domestic animals have been criticised because of the possible development of antibiotic resistant Salmonella and the potential dangers of residual antibiotics and vaccines in animal-derived food products for human consumption (Jung & Beuchat, 2000; Lailler *et al.,* 2002; Guntupalli *et al.,* 2007; Perron *et al.,* 2007). Furthermore, antibiotics given to animals, and closely related compounds used in human therapy, have been exerting selective pressure on their target bacteria for decades, and can generate a reservoir of antimicrobial resistant bacteria (Montagne *et al*., 2003). Antimicrobial-resistant bacteria in food animals threaten the efficacy of human drugs if antimicrobial-resistant bacteria or antimicrobial-resistance genes become incorporated into human bacterial populations (Antunes *et al*., 2003; De Oliveira *et al*., 2005). For these reasons, many countries, including the European Union, have begun phasing out growth promoting antibiotics in broiler diets (Humphrey, 2001; Wegener, 2003; Phillips, 2007). As a result, a number of alternative feed additives have been proposed to replace antibiotics, such as exogenous enzymes, probiotics, fermentable carbohydrates, zinc and dietary acidifiers, with varying and limited success (Dibner & Richards, 2005; Dahiya *et al.,* 2006).

Bacitracin (BC) is one of the most common antibiotics used in the world as an animal feed additive due to its growth-promoting effects, it is effective against Gram-positive organisms (Capitan-Vallvey *et al.,* 2002; Engberg *et al.,* 2000) and will therefore not inhibit Salmonella, which are Gram-negative organisms (Jordan & Pattison, 1996; Yeh *et al.,* 2002; Ehrbar & Hardt, 2005; Guntupalli *et al.,* 2007). The main site of antibiotic activity of Zinc-Bacitracin (Zn-BC) is within the gastrointestinal tract, where Zn-BC acts to modify the intestinal flora as well as the gut wall structure, (Huyghebaert & de Groote, 1997). The reason for the inclusion of Zn-BC in this trial was to determine whether inhibiting the growth of the gut microflora would allow the Salmonella to proliferate in the body of the chicken.

It is essential for the poultry production industry to develop new feed additives and processing techniques as alternatives for sub-therapeutic dietary supplementation of antibiotics. Innovative research is needed to evaluate existing products and to develop new ones.

The general aim of this trial was to determine the effects of *Salmonella typhimurium* colonisation of the gastrointestinal tract of broiler chicks on gastrointestinal health and production performance. In order to determine these effects, intestinal damage, organ weights, serum biochemical profile and performance parameters were measured. Histopathological sampling was also done on various organs. The effect of Zinc-Bacitracin, a commonly used AGP in the poultry industry, on Salmonella colonisation was also measured. The ultimate aim of the study was to obtain baseline values of various parameters that could be used in future trials for the evaluation of antibiotic alternative products.

The null hypothesis was that Salmonella colonisation of the gastrointestinal tract of broiler chicks does not have any quantifiable effects on the birds and therefore evaluating alternative products to control Salmonella infection is impossible. The alternative hypothesis is that colonisation will have quantifiable effects therefore making evaluation of alternative products possible.

4.1 Materials and methods

4.1.1) *Chickens*: A total of 2300 commercial Ross 788 broiler eggs were obtained from Eagle's Pride Hatchery (Pretoria, South Africa). The eggs were set at the hatchery facilities on the Research Farm of the University of Pretoria (Hatfield, Pretoria). The chicks were feather sexed at hatch. A total of 1680 first-grade chicks were selected and randomly placed into 42 pens with 40 chicks (20 males and 20 females) per pen. The chicks were screened for Salmonella by means of a faecal swab sample taken on day 1 of the trial.

4.1.2) *Experimental design:* The trial was conducted in two separate broiler facilities on the Research Farm of the University of Pretoria (Hatfield, Pretoria). One facility housed a Salmonella-free group, which were subdivided into two groups that received a diet containing either no antibiotic growth promoters or a sub-therapeutic level of zinc bacitracin (333 mg/kg feed). These Salmonella-free chicks received 0.2 mL of sterile saline solution orally on day four.

The second facility housed two treatment groups that were challenged with different Salmonella levels, namely 2×10^4 and 2×10^9 colony forming units (CFU). The chicks received 0.2 mL of the Salmonella suspension via oral gavage on day four. These two main groups were subdivided and each subgroup received a diet containing either no zinc bacitracin or zinc bacitracin at 333 mg/kg feed.

All treatments were replicated 7 times. On day one, 40 chicks were randomly allocated to each replicate. This was reduced on day four to a total of 30 chicks after weighing and removal of the

outliers to ensure a more uniform flock at the start of the trial when inoculation with Salmonella took place. Culling took place regardless of sex.

4.1.3) *Bacteria:* A culture of a Naladixic Acid Resistant strain of *Salmonella enterica* subsp. enterica serovar Typhimurium was obtained from the Veterinary Institute Onderstepoort, South Africa. The Salmonella suspension was administered on day four of the trial via oral gavage. The chicks received 0.2 mL of the Salmonella suspension at one of two concentrations, namely 2 x 10^4 CFU/mL and 2 x 10^9 CFU/mL. Chicks in the Salmonella-free control group received 0.2mL of sterile saline solution via oral gavage. The methods used in the culturing of the bacteria can be found in the Appendix.

4.1.4) *Husbandry*: The experiments were conducted in environmentally controlled broiler houses fitted with concrete floors and covered with wood shavings as bedding material. Each replicate of the various treatments were kept in similar pens with a surface area of 1.5m^2 and an open space of approximately 50cm between adjoining pens to prevent direct contact between chicks of different replicates. The pens were equipped with infra-red heating lamps, tube feeders, bell drinkers and fountain drinkers. Chicks received feed and water on an *ad libitum* basis. The houses were fumigated with formaldehyde gas after placement of the bedding material, 5 days before the arrival of the chicks. The temperature and ventilation of each of the facilities were closely monitored and regulated through the combined use of heating lamps and electrical fans. The temperature was initially kept at approximately 32 - 34ºC for the first two days after which it was gradually reduced by 2.8ºC per week. A lighting programme of 23 hours light and 1-hour darkness was employed.

4.1.5) *Diets:* The chicks were reared on a 3-phase diet. They received a starter diet from day 1 to 7, a grower diet from day 8 to 28 and a finisher diet from day 29 to 35. The composition of the diets is shown in Table 4.1, Table 4.2 and Table 4.3 respectively. No coccidiostats were included in the diets. All feeds were irradiated after mixing with 5kGy (Isotron South Africa, Kempton Park) to prevent Salmonella contamination of chicks via the feed. The feed was formulated using Format Software (Format International, UK). Each diet contained one of two antibiotic growth promoter levels, namely zero or a sub therapeutic level of 333 mg zinc bacitracin per kg of feed.

4.1.6) *Measurements, sampling and sample analysis*: Cloacal swabs from 10 chicks per pen were tested weekly for the presence of Salmonella. The swabs were enriched in Rappaport-Vassiliadis broth incubated over-night and plated onto Rambach Agar at the Department of Microbiology and Plant Pathology, University of Pretoria. On days 7, 14, 21, 28 and 35 of the trial three chicks per replicate were sacrificed by cervical dislocation. Their gastrointestinal tracts (GIT) were examined for intestinal lesions and the duodenum, jejenum and ileum of these chicks were removed and stored for villous morphological measurements. Blood samples were collected at each slaughter and centrifuged to obtain the serum that was analysed for its biochemical profile at the Department of Clinical Pathology, Onderstepoort, University of Pretoria.

a) Intestinal damage: Immediately after sacrifice the duodenum, jejenum and ileum were opened by a longitudinal incision along the antimesenteric side and cleaned of their contents in saline solution. They were examined for lesions with the length and width of each lesion being measured using a binocular lens. The extent was measured using a modified version of the scale used by Villegas *et al* (2001): 0 = absence of haemorrhage, 1 = slight haemorrhage, 2 = moderate haemorrhage and 3 = severe haemorrhage.

Table 4.1 Raw material composition and nutrient levels of the starter diet

Ingredient	**% Inclusion**
Yellow maize	59.6
Soya oil cake 47%	26.4
Local fish meal 65%	11.0
Monocalcium phosphate	1.29
Limestone 36%	1.07
Premix	0.50
Salt	0.15
Calculated Nutrient Levels	**g/kg**
ME	12.7 MJ/kg
Crude protein	243
Lysine	14.7
Methionine	4.80
Calcium	11.0
Available phosphorous	5.10
Sodium	1.66
Fat	38.8
Fibre	26.9

b) Villous morphological measurements: Cross sections of the duodenum, jejenum and ileum samples that were preserved in 10% Millonig's Buffered Formalin solution were imbedded according to standard imbedding practices. Samples were dehydrated in an ethanol: distilled-water series (30:70; 50:50; 70:30; 100:0; 100:0) followed by a xylene: ethanol series (30:70; 50:50; 70:30; 100:0; 100:0). The samples were then imbedded in paraffin wax (melting point 60ºC). Samples were sectioned at 8µm thickness and stained with Haematoxylin and Eosin (H&E). Villous height and crypt depth were measured on the stained sections using a Nikon digital camera DXM1200F light microscope with 20x combined magnification and an ocular micrometer for the sections taken on day 14 of age, and 10x combined magnification for the sections taken on day 28 of age.

c) Organ weights: The weights of the liver, spleen, duodenum, jejenum, ileum and caeca were recorded and expressed as a percentage of body weight at the time of slaughter.

d) Histological examination: The bursa of Fabricius, caeca, and liver were sent for histological examination at the Pathology Laboratory of the Faculty of Veterinary Science, Onderstepoort, University of Pretoria. They were imbedded following standard imbedding practices and stained with Haematoxylin and Eosin (H&E).

Table 4.2 Raw material composition and nutrient levels of the grower diet

Ingredient	**% Inclusion**
Yellow maize	64.8
Soya oil cake 47%	15.7
Local fish meal 65%	10.0
Full fat Soya	6.74
Monocalcium phosphate	1.19
Limestone 36%	0.84
Premix	0.50
Salt	0.19
Calculated Nutrient Levels	**g/kg**
ME	13.22 MJ/kg
Crude protein	215
Lysine	12.7
Methionine	4.37
Calcium	9.50
Available phosphorous	4.70
Sodium	1.70
Fat	50.0
Fibre	27.3

e) Broiler performance: Live chick body weights and feed intake were recorded for each pen on a weekly basis. Feed wastage and spillage was kept to a minimum with frequent monitoring. Feed conversion ratios were calculated and mortality was recorded as it occurred.

f) Serum biochemical profile: Total serum protein (TSP), aspartate transaminase activity (AST) and albumin and globulin levels in the serum were determined by the Department of Clinical Pathology Onderstepoort, University of Pretoria.

Serum albumin

Serum samples were collected for albumin analyses. Albumin concentration was measured on a TECHNICON RA-1000® system (Miles Inc., Diagnostics Division, Tarrytown, New York, USA) according to standard procedures, as explained in the Technicon RA Systems Manual (Method No. SM4-0131E94, May 1994). This albumin method is based on the work of Doumas *et al*. (1971) who automated the original manual method of Rodkey (1965).

Total Serum Protein (TSP) - Serum samples were collected for TSP analyses. TSP concentrations were measured on a TECHNICON RA-1000® system (Miles Inc., Diagnostics Division, Tarrytown, New York, USA) according to standard procedures, as explained in the Technicon RA Systems Manual (Method No. SM4-0147E94, May 1994). This total method is based on the work of Skeggs & Hochstrasser (1964) who automated the manual method of Weichselbaum (1946).

Table 4.3 Raw material composition and nutrient levels of the finisher diet

Ingredient	**% Inclusion**
Yellow maize	71.0
Soya oil cake 47%	13.5
Local fish meal 65%	8.20
Full fat Soya	4.50
Monocalcium phosphate	1.00
Limestone 36%	1.00
Premix	0.50
Salt	0.25
Calculated Nutrient Levels	**g/kg**
ME	13.33 MJ/kg
Crude protein	190
Lysine	10.8
Methionine	3.88
Calcium	9.00
Available phosphorous	4.03
Sodium	1.78
Fat	46.4
Fibre	26.7

Globulin – Serum globulin values were calculated as the difference between TSP and albumin.

Aspartate Aminotransferase (AST) - Serum samples were collected for AST analyses. AST concentrations were measured on a TECHNICON RA-1000® system (Miles Inc., Diagnostics Division, Tarrytown, New York, USA) according to standard procedures, as explained in the Technicon RA® Systems Manual (Method No. SM4-0137E94, May 1994). The Technicon RA® system AST method is based on work by Karmen (1955) who originated a procedure that coupled malate dehydrogenase and NADH to the aminotransferase reaction. Bergmeyer *et al*. (1978) modified this procedure to eliminate side reactions and to optimize substrate conditions.

4.1.7) Statistical Analysis

An analysis of variance with the GLM model (Statistical Analysis Systems, 2001) was used to determine the significance between different treatment levels and the interaction between treatments

and levels. Means and standard deviations were calculated. Significance of difference (5%) between means was determined using Bonferroni's test. Chi-Square analysis was done to evaluate intestinal damage using a significance level of 0.05.

4.2 Results

4.2.1 Organ weights

As shown in Tables 4.4 – 4.7, exposure to Salmonella caused an increase in the weights of the duodenum, jejunum, ileum and caeca during the first week after inoculation, where after the effect disappears within the next week. This effect was notable regardless whether the birds received zinc bacitracin in their diets or not. On day 28, Salmonella-exposed broilers had significantly larger livers than the non-exposed broilers (Table 4.8).

4.2.2 Broiler performance

Groups exposed to Salmonella that did not receive antibiotics had significantly higher body weights for the first 3 weeks, where after the effect disappeared (Table 4.9). The same effect was noted for average daily gain of the birds, with birds exposed to Salmonella and receiving no antibiotics having higher ADG than the control birds for the first 3 weeks (Table 4.11). Noteworthy was the apparent effect of the presence of zinc bacitracin in the feed on growth. Zinc bacitracin had no effect on body weight in the control (non-exposed) birds. However, where birds were exposed to Salmonella, zinc bacitracin clearly inhibited growth. This effect was significant during the early growth stage, but evident throughout the duration of the trial.

Feed intake increased shortly after the birds were inoculated with ST, but this effect was not clear from day 14 onwards (Table 4.10).

Exposure to low levels of ST viz. 2 x 10^4 CFU/mL led to higher feed conversion ratio in those birds receiving antibiotics than in those that did not receive antibiotics in the second, third and fourth week, where after the effect disappeared (Table 4.12).

Only one mortality occurred in the control birds in week 3 for the group that received antibiotics. Exposure to the low level of ST viz. 2 x 10^4 CFU/mL combined with antibiotics resulted in three mortalities, with two occurring in week two and one occurring in week three. Exposure to higher levels of ST viz. 2 x 10^9 CFU/mL resulted in one mortality in week four in the group that did not receive antibiotics.

4.2.3 Serum biochemical profile

Albumin levels were lower shortly after the birds were challenged with ST. From day 14, this effect could no longer be detected (Table 4.13).

Globulin levels were significantly decreased at day 7 in broilers inoculated with the lower level of Salmonella. However, for the higher ST level at day 7, and both ST levels at day 14, ST exposure caused broilers to have increased levels of serum globulin (Table 4.14). Total protein levels in the serum followed the same pattern than the globulin levels (Table 4.16).

Aspartate transaminase activity measurements showed no significant results regardless of Salmonella and antibiotic level (Table 4.17). However, there was a general trend for increased levels of AST during the first two weeks after Salmonella inoculation.

Table 4.4 Duodenum weights expressed as percentage of body weight measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	1.80 (±0.301)^{a}	1.91 (±0.344)^{x}	2.33 (±0.376)^{b}	2.34 (±0.295)^{xy}	2.51 (±0.424)^{b}	2.41 (±0.246)^{y}
14	2.06 (±0.211)	2.12 (±0.201)^{x}	1.87 (±0.081)	1.79 (±0.110)^{xy}	1.99 (±0.221)	1.98 (±0.125)^{y}
21	1.46 (±0.109)	1.51 (±0.110)	1.54 (±0.173)	1.48 (±0.131)	1.60 (±0.161)	1.51 (±0.093)
28	1.10 (±0.063)^{a}	1.10 (±0.078)^{x}	1.23 (±0.047)^{b}	1.20 (±0.076)^{y}	1.21 (±0.090)^{b}	1.18(±0.071)^{y}
35	0.85 (±0.066)	0.88 (±0.095)	0.91 (±0.091)	0.84 (±0.048)	0.90 (±0.059)	0.90 (±0.098)

^{ab}Row means with the same superscript within AB treatments between Salmonella levels do not differ significantly (P > 0.016)
^{xy}Row means with the same superscript within NA treatments between Salmonella levels do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.5 Jejenum weights expressed as percentage of body weight measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	1.78 (±0.360)^{a}	2.02 (±0.448)	2.56 (±0.753)^{b}	2.54 (±0.629)	2.76 (±0.536)^{b}	2.67 (±0.555)
14	2.26 (±0.156)	2.44 (±0.128)^{x}	2.27 (±0.137)	2.10 (±0.168)^{y}	2.28 (±0.142)	2.31 (±0.284)^{xy}
21	1.86 (±0.143)	1.83 (±0.063)	1.89 (±0.181)^{1}	1.66 (±0.218)^{2}	1.86 (±0.094)	1.77 (±0.094)
28	1.54 (±0.099)	1.48 (±0.138)	1.59 (±0.154)	1.55 (±0.179)	1.58 (±0.113)	1.51 (±0.138)
35	1.08 (±0.056)	1.19 (±0.075)^{x}	1.17 (±0.135)^{1}	1.04 (±0.132)^{2y}	1.09 (±0.152)^{1}	1.22 (±0.071)^{2x}

^{12}Row means with the same superscript within the same Salmonella level do not differ significantly (P > 0.05)
^{ab}Row means with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
^{xy}Row means with the same superscript within the same level (NA) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.6 Ileum weights expressed as percentage of body weight measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	1.63 (±0.331)^{a}	1.58 (±0.292)^{x}	2.28 (±0.474)^{b}	2.03 (±0.433)^{xy}	2.39 (±0.451)^{b}	2.40 (±0.409)^{y}
14	1.95 (±0.282)	1.95 (±0.108)	2.06 (±0.165)	1.84 (±0.188)	1.95 (±0.110)	1.94 (±0.395)
21	1.53 (±0.083)	1.58 (±0.149)	1.58 (±0.084)	1.57 (±0.148)	1.56 (±0.111)	1.45 (±0.138)
28	1.35 (±0.111)	1.31 (±0.192)	1.28 (±0.090)	1.22 (±0.148)	1.21 (±0.087)	1.25 (±0.104)
35	0.87 (±0.033)	0.93 (±0.082)^{xy}	0.95 (±0.117)	0.83 (±0.107)^{x}	0.96 (±0.106)	0.98 (±0.081)^{y}

^{ab}Row means with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
^{xy}Row means with the same superscript within the same level (NA) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.7 Caeca weights expressed as percentage of body weight measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2×10^4 CFU Salmonella		2×10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	0.76 (±0.210)[a]	0.79 (±0.207)	1.15 (±0.177)[b]	0.96 (±0.292)	1.10 (±0.274)[b]	1.08 (±0.226)
14	0.74 (±0.122)	0.68 (±0.055)	0.73 (±0.097)	0.66 (±0.059)	0.64 (±0.120)	0.74 (±0.078)
21	0.60 (±0.067)	0.60 (±0.104)	0.65 (±0.088)	0.64 (±0.082)	0.68 (±0.058)	0.62 (±0.063)
28	0.53 (±0.039)	0.50 (±0.047)	0.52 (±0.060)	0.53 (±0.051)	0.59 (±0.054)	0.53 (±0.073)
35	0.38 (±0.032)	0.42 (±0.017)	0.38 (±0.058)	0.37 (±0.064)	0.38 (±0.037)	0.39 (±0.043)

[ab]Row means with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.8 Liver weights expressed as percentage of body weight measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	4.88 (±0.177)	4.77 (±0.262)	5.05 (±0.499)	4.83 (±0.380)	4.97 (±0.391)	5.02 (±0.330)
14	3.48 (±0.310)	3.33 (±0.199)	3.50 (±0.612)	3.32 (±0.283)	3.89 (±0.393)[1]	3.25 (±0.299)[2]
21	3.22 (±0.210)	3.08 (±0.234)	3.35 (±0.465)	3.25 (±0.239)	3.30 (±0.457)	3.36 (±0.411)
28	3.24 (±0.234)[a]	3.20 (±0.226)[x]	3.70 (±0.564)[ab]	3.60 (±0.182)[xy]	3.86 (±0.542)[b]	3.74 (±0.306)[y]
35	2.95 (±0.231)	2.87 (±0.266)	2.84 (±0.213)	2.89 (±0.269)	2.92 (±0.159)	2.90 (±0.100)

[12]Row means with the same superscript within the same Salmonella level do not differ significantly (P > 0.05)
[ab]Row means with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
[xy]Row means with the same superscript within the same level (NA) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.9 Body weight (g) measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
0	40.42 (±0.965)	40.23 (±0.937)	39.8 (±1.712)	39.6 (±0.751)	39.58 (±1.061)	40.34 (±1.394)
7	120.2 (±3.176)	118.1 (±4.412)^{x}	123.3(±5.941)^{1}	130.5 (±6.704)^{2y}	119.9 (±5.714)^{1}	127.3 (±7.158)^{2y}
14	339.1 (±7.823)^{a}	339.0 (±10.91)^{x}	324.8 (±23.41)^{1ab}	362.6 (±19.99)^{2y}	314.3 (±8.724)^{1b}	343.5 (±22.75)^{2xy}
21	617.1 (±27.53)	628.1 (±17.26)^{x}	618.0 (±42.46)^{1}	672.2 (±24.85)^{2y}	612.9 (±8.743)	641.8 (±30.70)^{xy}
28	1115 (±41.23)	1143 (±38.83)	1105 (±51.74)	1143 (±42.78)	1099 (±40.23)	1116 (±42.34)
35	1743 (±71.57)	1702 (±72.34)	1698 (±64.38)	1714 (±83.42)	1736 (±57.66)	1744 (±57.74)
Cumulative (day 0-35)	1785 (±172.1)	1797 (±76.19)	1741 (±153.4)	1804 (±107.0)	1632 (±13.31)	1686 (±141.8)

^{12}Row means with the same superscript within the same Salmonella level do not differ significantly (P > 0.05)
^{ab}Row means with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
^{xy}Row means with the same superscript within the same level (NA) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.10 Weekly feed intake (g) measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	76.63 (±2.065)^{a}	73.35 (±3.241)^{x}	82.25 (±5.593)^{ab}	87.51 (±6.080)^{y}	84.59 (±7.247)^{b}	85.17 (±5.881)^{y}
14	435.5 (±4.047)	434.6 (±3.765)	456.6 (±48.94)	439.4 (±1.775)	438.5 (±2.654)	438.6 (±2.536)
21	538.7 (±22.58)	575.8 (±71.00)	545.0 (±17.87)	532.5 (±56.84)	547.1 (±15.13)	556.1 (±4.678)
28	840.6 (±73.60)	813.1 (±38.87)	840.7 (±77.85)	792.1 (±48.11)	863.3 (±78.29)	863.1 (±56.40)
35	1020 (±63.39)	1002 (±75.17)	962.2 (±75.19)	1019 (±20.91)	983.4 (±106.8)	1029 (±71.59)
Cumulative (day 0-35)	2949 (±142.3)	2935 (±86.98)	2924 (±167.8)	2911 (±87.86)	2953 (±189.3)	3011 (±119.0)

^{ab}Row means with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
^{xy}Row means with the same superscript within the same level (NA) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.11 Average daily gain (g) measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	11.39 (±11.390)	11.12 (±11.120)^{x}	11.93 (±11.932)	12.98 (±12.981)^{y}	11.47 (±0.799)^{1}	12.42 (±0.947)^{2y}
14	21.33 (±21.334)^{a}	21.34 (±21.338)^{x}	20.36 (±20.356)^{1ab}	23.07 (±23.072)^{2y}	19.63 (±0.635)^{1b}	21.66 (±1.607)^{2xy}
21	27.46 (±27.460)	27.99 (±27.993)^{x}	27.53 (±27.535)^{1}	30.12 (±30.122)^{2y}	27.30 (±0.428)	28.64 (±1.453)^{xy}
28	38.41 (±38.409)	39.42 (±39.418)	38.06 (±38.056)	39.43 (±39.429)	37.85 (±1.412)	38.43 (±1.477)
35	48.65 (±48.648)	47.50 (±47.497)	47.36 (±47.364)	47.86 (±47.856)	48.48 (±1.638)	48.68 (±1.653)
Cumulative (day 0-35)	49.85 (±4.908)	50.22 (±2.160)	48.63 (±4.379)	50.41 (±3.057)	45.49 (±4.945)	47.03 (±4.057)

^{12}Row means with the same superscript within the same Salmonella level do not differ significantly (P > 0.05)
^{ab}Row means with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
^{xy}Row means with the same superscript within the same level (NA) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.12 Weekly feed conversion ratio (g feed / g body weight gain) measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	0.95 (±0.030)	0.94 (±0.026)	0.97 (±0.045)	0.98 (±0.051)	1.01 (±0.074)	0.97 (±0.068)
14	1.62 (±0.039)^{a}	1.61 (±0.055)	1.78 (±0.173)^{1b}	1.57 (±0.080)^{2}	1.78 (±0.063)^{1b}	1.64 (±0.106)^{2}
21	1.77 (±0.080)	1.78 (±0.099)^{x}	1.81 (±0.078)^{1}	1.64 (±0.089)^{2y}	1.81 (±0.046)	1.75 (±0.080)^{x}
28	1.73 (±0.135)	1.69 (±0.082)	1.78 (±0.119)^{1}	1.66 (±0.110)^{2}	1.79 (±0.105)	1.78 (±0.097)
35	1.69 (±0.123)	1.73 (±0.098)	1.73 (±0.156)	1.70 (±0.128)	1.70 (±0.115)	1.73 (±0.077)
Cumulative (day 0-35)	1.71(±0.197)	1.67 (±0.107)	1.73 (±0.218)	1.66 (±0.119)	1.88 (±0.262)	1.84 (±0.185)

^{12}Row means with the same superscript within the same Salmonella level do not differ significantly (P > 0.05)
^{ab}Row means with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
^{xy}Row means with the same superscript within the same level (NA) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.13 Albumin levels (g/L) measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	17.63 (±2.349)[a]	16.57 (±1.957)	14.45 (±1.631)[b]	14.24 (±1.905)	16.50 (±1.763)[ab]	15.51 (±1.619)
14	14.93 (±1.121)	14.84 (±0.993)	15.46 (±0.950)	16.20 (±0.753)	16.19 (±1.588)	16.54 (±1.926)
21	17.86 (±1.288)	17.64 (±0.565)	16.23 (±1.628)	15.99 (±1.737)	15.81 (±2.230)	16.66 (±2.337)
28	16.69 (±1.161)	16.47 (±0.789)	15.89 (±1.259)	16.13 (±0.850)	15.93 (±0.820)	16.43 (±0.820)
35	16.71 (±0.689)	16.41 (±0.687)	16.54 (±0.658)	17.59 (±1.401)	16.71 (±1.019)	16.86 (±1.257)

[ab]Row means with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.14 Globulin levels (g/L) measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	13.60 (±2.682)^{a}	12.01 (±2.362)	10.27 (±2.593)^{b}	9.39 (±2.125)	12.53 (±2.519)^{b}	12.00 (±2.176)
14	9.04 (±1.468)	8.81 (±1.085)^{x}	10.71 (±1.843)	10.59 (±1.515)^{xy}	11.79 (±3.101)	12.96 (±3.425)^{y}
21	12.04 (±1.288)	12.14 (±0.591)	11.31 (±1.975)	11.61 (±1.824)	12.16 (±1.370)	13.13 (±1.597)
28	13.94 (±1.672)	13.91 (±1.436)	12.67 (±1.925)	12.86 (±2.551)	11.87 (±1.147)	12.84 (±2.012)
35	11.93 (±1.238)	12.90 (±1.828)	10.84 (±1.685)^{1}	13.34 (±2.562)^{2}	10.66 (±1.913)	12.66 (±1.999)

^{12}Row means with the same superscript within the same Salmonella level do not differ significantly (P > 0.05)
^{ab}Row means with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
^{xy}Row means with the same superscript within the same level (NA) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.15 Albumin:Globulin ratio measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	1.32 (±0.199)	1.41 (±0.227)	1.47 (±0.353)	1.58 (±0.375)	1.35 (±0.194)	1.32 (±0.215)
14	1.67 (±0.177)	1.70 (±0.138)^{x}	1.46 (±0.163)	1.55 (±0.210)^{x}	1.43 (±0.248)	1.32 (±0.246)^{y}
21	1.49 (±0.101)	1.46 (±0.089)	1.46 (±0.215)	1.41 (±0.305)	1.30 (±0.150)	1.28 (±0.192)
28	1.20 (±0.073)	1.19 (±0.086)	1.27 (±0.127)	1.29 (±0.216)	1.35 (±0.082)	1.30 (±0.191)
35	1.41 (±0.105)	1.29 (±0.182)	1.55 (±0.183)	1.36 (±0.247)	1.60 (±0.243)^{1}	1.36 (±0.186)^{2}

[12]Row means with the same superscript within the same Salmonella level do not differ significantly (P > 0.05)
[xy]Row means with the same superscript within the same level (NA) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.16 Total serum protein levels (g/L) measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	31.23 (±4.690)^{a}	28.59 (±4.010)	24.73 (±3.522)^{b}	23.63 (±3.164)	29.03 (±4.051)^{ab}	27.51 (±3.338)
14	23.97 (±2.536)	23.66 (±2.016)^{x}	26.17 (±2.778)	26.79 (±2.017)^{xy}	27.97 (±4.553)	29.50 (±4.976)^{y}
21	29.90 (±2.432)	29.79 (±0.811)	27.54 (±3.176)	27.60 (±2.285)	27.97 (±3.292)	29.79 (±3.175)
28	30.63 (±2.784)	30.39 (±2.118)	28.56 (±3.019)	28.99 (±3.310)	27.80 (±1.888)	29.27 (±2.504)
35	28.64 (±1.867)	29.31 (±1.989)	27.39 (±2.279)^{1}	30.93 (±3.093)^{2}	27.40 (±2.715)	29.51 (±2.957)

^{12}Row means with the same superscript within the same Salmonella level do not differ significantly (P > 0.05)
^{ab}Row means with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
^{xy}Row means with the same superscript within the same level (NA) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.17 Aspartate transaminase levels (IU/L) measured at days 7, 14, 21, 28 and 35 of age for broilers challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
7	223.7 (±42.15)[1]	517.6 (±359.1)[2]	176.6 (±29.30)	234.4 (±88.45)	360.7 (±383.9)	238.3 (±99.16)
14	178.7 (±23.27)	193.1 (±37.5)	433.3 (±274.5)	614.1 (±772.0)	370.3 (±245.1)	570.0 (±300.2)
21	189.0 (±20.03)	206.4 (±54.07)	187.9 (±33.32)	189.4 (±18.17)	173.6 (±12.54)	193.6 (±20.29)
28	193.4 (±7.656)	194.6 (±26.93)	176.1 (±9.940)	174.6 (±14.86)	186.7 (±30.71)	177.9 (±5.242)
35	206.7 (±24.60)	204.1 (±19.85)	211.7 (±23.91)	211.6 (±16.27)	198.6 (±14.57)	201.6 (±31.78)

[12]Row means with the same superscript within the same Salmonella level do not differ significantly (P > 0.05)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

4.2.4 Intestinal damage

No lesions were found in any of the birds slaughtered at day 7 and day 14 of age, while lesions did occur in the birds slaughtered at day 21, 28 and 35 of age. Chi-Square analysis was done for the number of broilers presented with lesions on day 21, 28 and 35 (Tables 4.18 - 4.20). Using a significance level of 0.05, it was found that there were no statistically significant differences between treatment groups for the number of chicks in which lesions occurred. However, there were consistently more chickens that had intestinal lesions when they were infected with Salmonella.

Table 4.18 The number of chicks that had gastrointestinal lesions at day 21 of age according to *Salmonella typhimurium* (ST) level and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

ST level (CFU/mL)	Antibiotic	No antibiotic
0	9	6
2 x 10^4	14	15
2 x 10^9	15	12

*Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.19 The number of chicks that had gastrointestinal lesions at day 28 of age according to *Salmonella typhimurium* (ST) level and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

ST level (CFU/mL)	Antibiotic	No antibiotic
0	2	3
2 x 10^4	15	15
2 x 10^9	14	19

*Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.20 The number of chicks that had gastrointestinal lesions at day 35 of age according to *Salmonella typhimurium* (ST) level and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

ST level (CFU/mL)	Antibiotic	No antibiotic
0	2	3
2 x 10^4	17	21
2 x 10^9	18	20

*Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.21, Table 4.22 and Table 4.23 were based on the pen averages for the severity of lesions on a scale of 0 = no lesions, 1 = mild lesions, 2 = moderate lesions and 3 = severe lesions.

Table 4.21 Gastrointestinal lesion scores based on pen average for birds slaughtered at day 21 of age and challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

ST level (CFU/mL)	Antibiotic	No antibiotic
0	0.38 (±0.405)	0.67 (±0.745)
2 x 10^4	0.86 (±0.424)	0.76 (±0.252)
2 x 10^9	0.81 (±0.573)	0.81 (±0.424)

* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.22 Gastrointestinal lesion scores* based on pen average for birds slaughtered at day 28 of age and challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

ST level (CFU/mL)	Antibiotic	No antibiotic
0	0.14 (±0.178)[a]	0.10 (±0.162)[a]
2 x 10^4	1.10 (±0.686)[b]	1.24 (±0.659)[b]
2 x 10^9	1.24 (±0.460)[b]	0.90 (±0.460)[b]

* Scale used: 0 = absence of haemorrhage, 1 = slight haemorrhage, 2 = moderate haemorrhage and 3 = severe haemorrhage.

[ab]Column means with the same superscript do not differ significantly (P > 0.016)

* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.23 Gastrointestinal lesion scores* based on pen average for birds slaughtered at day 35 of age and challenged with different levels of *Salmonella typhimurium* (ST) and either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet (value ± standard deviation of the mean)

ST level (CFU/mL)	Antibiotic	No antibiotic
0	0.14 (±0.178)[a]	0.10 (±0.162)[a]
2 x 10^4	1.95 (±0.621)[b]	1.90 (±0.937)[b]
2 x 10^9	1.76 (±0.317)[b]	1.52 (±0.716)[b]

* Scale used: 0 = absence of haemorrhage, 1 = slight haemorrhage, 2 = moderate haemorrhage and 3 = severe haemorrhage.

[ab]Column means with the same superscript do not differ significantly (P > 0.016)

* Antibiotics included as zinc bacitracin at 333 mg/kg feed

The lesion data results for the birds slaughtered on days 28 and 35 of age (Table 4.22 and Table 4.23) showed that the inclusion of zinc bacitracin in the feed did not affect the severity of intestinal lesions. However, there was a significant difference in lesion scoring between the control birds and the Salmonella infected birds regardless of antibiotic inclusion, with the control birds having lower lesion scores than the groups inoculated with Salmonella.

4.2.5 Histopathology

Histopathology results gave no conclusive evidence of any effects of Salmonella or antibiotic level on the caeca (Table 4.24 – 4.28). The same inconclusive results were found for histopathological studies on the bursa and livers of the broilers (results not shown). For the first slaughter, all of the liver results were classifies as “nothing abnormal or nothing remarkable” (NA/NR), while six bursal samples showed signs of mild necrosis. For the second slaughter, four liver samples showed signs of multifocal lymph aggregates while two bursal samples showed signs of grade1 and grade 3 necrosis, respectively. For the third slaughter, two liver samples showed signs of multifocal lymph aggregates, while 1 bursal sample showed signs of grade1 necrosis. For the fourth slaughter, all of the liver samples were NA/NR while seven bursal samples showed signs of necrosis with the presence of large quantities of bacteria. For the fifth slaughter, three liver samples showed signs of multifocal lymph aggregates, while nine bursal samples showed signs of the presence of large quantities of bacteria.

4.2.6 Villous morphological measurements

As shown in Tables 4.29 – 4.34, no significant differences were found between the treatments for villi measurements taken from the birds slaughtered at day 14 of age. Villi measurements taken at day 28 of age showed some significant differences. There was a significant difference between antibiotic groups that were inoculated with 2 x 10^9 CFU ST/mL, with those birds receiving antibiotics having longer duodenal villi lengths than those that did not receive antibiotics. On day 28 of age, the duodenal and jejenal villi lengths of birds that were challenged with Salmonella, were shorter than in those birds that were not challenged. This trend was also noted for villi length in the ileum of birds challenged with ST that received zinc bacitracin in their feed.

Table 4.24 The number of birds, challenged with different levels of *Salmonella typhimurium* (ST) and receiving different antibiotic levels, showing caecal histopathology after slaughtered at day 7 of age

Effect seen	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
NA/NR	7	5	5	12	12	12
Slough	9	10	8	4	2	4
Presence of bacteria	7	16	14	5	6	4
MFN	1	8	5	2	1	3
MFJN	0	0	0	0	0	0
Heterophils	0	0	0	0	3	3
Separation	0	0	2	3	6	2
Necrosis	0	1	3	0	0	0
Infiltrate	0	0	0	0	1	0
Mucosa gone	3	1	0	0	0	0

NA/NR = nothing abnormal/ nothing remarkable

MFN = multifocal necrosis

MFJN = multifocal junctional necrosis

Table 4.25 The number of birds, challenged with different levels of *Salmonella typhimurium* (ST) and receiving different antibiotic levels, showing caecal histopathology after slaughtered at day 14 of age

Effect seen	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
NA/NR	2	2	6	3	2	3
Slough	2	5	1	1	5	7
Presence of bacteria	2	8	7	5	4	5
MFN	10	10	8	10	9	9
MFJN	16	12	8	15	15	11
Heterophils	1	2	0	2	1	3
Separation	1	5	5	6	6	8
Necrosis	0	0	1	1	3	2

NA/NR = nothing abnormal/ nothing remarkable

MFN = multifocal necrosis

MFJN = multifocal junctional necrosis

Table 4.26 The number of birds, challenged with different levels of *Salmonella typhimurium* (ST) and receiving different antibiotic levels, showing caecal histopathology after slaughtered at day 7 of age

Effect seen	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
NA/NR	5	4	0	2	4	2
Slough	1	2	5	1	4	5
Presence of bacteria	2	2	2	7	6	6
MFN	9	7	12	6	9	7
MFJN	13	14	14	17	15	16
Heterophils	0	1	2	2	3	1
Separation	0	1	2	11	5	7
Necrosis	2	3	2	8	1	2

NA/NR = nothing abnormal/ nothing remarkable

MFN = multifocal necrosis

MFJN = multifocal junctional necrosis

Table 4.27 The number of birds, challenged with different levels of *Salmonella typhimurium* (ST) and receiving different antibiotic levels, showing caecal histopathology after slaughtered at day 28 of age

Effect seen	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
NA/NR	1	2	4	0	1	1
Slough	10	10	8	11	8	8
Presence of bacteria	8	6	4	5	4	11
MFN	5	8	10	10	10	16
MFJN	8	9	9	10	8	12
Heterophils	2	3	0	0	0	1
Separation	5	5	7	5	4	5
Necrosis	0	0	0	2	2	5

NA/NR = nothing abnormal/ nothing remarkable

MFN = multifocal necrosis

MFJN = multifocal junctional necrosis

Table 4.28 The number of birds, challenged with different levels of *Salmonella typhimurium* (ST) and receiving different antibiotic levels, showing caecal histopathology after slaughtered at day 35 of age

Effect seen	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
NA/NR	0	0	0	0	1	0
Slough	14	11	17	20	15	17
Presence of bacteria	8	6	8	4	7	7
MFN	5	8	0	0	0	0
MFJN	3	6	0	0	0	0
Heterophils	0	0	0	0	0	0
Separation	1	4	0	0	0	0
Necrosis	3	7	10	5	6	7

NA/NR = nothing abnormal/ nothing remarkable

MFN = multifocal necrosis

MFJN = multifocal junctional necrosis

Table 4.29 Length of villi found in the duodenum and measured in µm for broilers slaughtered at day 14 and day 28 of age and challenged with different levels of *Salmonella typhimurium* (ST) while either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
14	649.2 (±137.1)	497.6 (±201.4)	535.4 (±71.29)	604.7 (±149.1)	501.8 (±158.5)	577.8 (±116.4)
28	719.2 (±44.91)^{a}	662.5 (±56.79)^{x}	592.0 (±49.75)^{b}	558.9 (±77.74)^{y}	637.9 (±37.95)^{1ab}	537.0 (±51.66)^{2y}

^{12}Row means that with the same superscript within the same Salmonella level do not differ significantly (P > 0.05)
^{ab}Row means that with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
^{xy}Row means that with the same superscript within the same level (NA) do not differ significantly (P > 0.016)
* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.30 Width of villi found in the duodenum and measured in µm for broilers slaughtered at day 14 and day 28 of age and challenged with different levels of *Salmonella typhimurium* (ST) while either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
14	70.43 (±27.82)	76.51 (±17.97)	72.16 (±7.576)	81.11 (±32.46)	69.09 (±38.50)	82.75 (±23.31)
28	154.7 (±19.77)	144.4 (±11.35)	116.5 (±20.91)	133.7 (±20.35)	125.1 (±31.86)	124.2 (±21.27)

* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.31 Length of villi found in the jejenum and measured in µm for broilers slaughtered at day 14 and day 28 of age and challenged with different levels of *Salmonella typhimurium* (ST) while either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
14	533.0 (±57.10)	500.3 (±105.7)	536.4 (±43.36)	571.8 (±121.1)	483.7 (±113.6)	528.4 (±60.39)
28	725.7 (±90.92)[a]	716.4 (±55.50)	615.3 (±69.26)[ab]	629.0 (±52.36)	557.2 (±124.5)[b]	595.2 (±60.92)

[ab]Row means that with the same superscript within the same level (AB) do not differ significantly (P > 0.016)
[*] Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.32 Width of villi found in the jejenum and measured in µm for broilers slaughtered at day 14 and day 28 of age and challenged with different levels of *Salmonella typhimurium* (ST) while either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
14	76.95 (±35.04)	85.67 (±26.34)	71.77 (±9.32)	69.90 (±15.96)	80.25 (±20.56)	86.04 (±24.96)
28	144.6 (±40.77)	144.7 (±27.34)	106.40 (±25.56)	116.7 (±28.49)	111.8 (±8.20)	107.2 (±16.25)

* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.33 Length of villi found in the ileum and measured in µm for broilers slaughtered at day 14 and day 28 of age and challenged with different levels of *Salmonella typhimurium* (ST) while either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet

Day	Treatment					
	0 CFU Salmonella		2 x 10^4 CFU Salmonella		2 x 10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
14	454.2 (±121.8)	566.8 (±206.3)	483.5 (±72.15)	574.6 (±156.9)	514.1 (±217.4)	515.9 (±125.5)
28	595.8 (±35.97)	530.3 (±81.26)	558.5 (±64.06)	541.1 (±56.76)	517.6 (±40.89)	534.2 (±84.84)

* Antibiotics included as zinc bacitracin at 333 mg/kg feed

Table 4.34 Width of villi found in the ileum and measured in µm for broilers slaughtered at day 14 and day 28 of age and challenged with different levels of *Salmonella typhimurium* (ST) while either receiving antibiotics (AB)* or receiving no antibiotics (NA) in their diet

Day	Treatment					
	0 CFU Salmonella		2×10^4 CFU Salmonella		2×10^9 CFU Salmonella	
	AB	NA	AB	NA	AB	NA
14	89.13 (±21.26)	88.42 (±13.75)	58.48 (±9.100)	73.38 (±21.29)	67.05 (±39.16)	79.96 (±15.08)
28	140.5 (±13.01)	124.4 (±17.34)	125.7 (±47.57)	131.7 (±18.10)	124.3 (±11.38)	121.0 (±11.01)

* Antibiotics included as zinc bacitracin at 333 mg/kg feed

4.3 Discussion

Cloacal swabs from non-challenged birds that received antibiotics and from the non-challenged birds that did not receive antibiotics tested negative for Salmonella, while the swabs from the challenged birds that received no antibiotics and from the challenged birds that did receive antibiotics tested positive. The biosecurity measures employed were thus effective in keeping the non-challenged birds free from Salmonella, and the antibiotic used did not inhibit Salmonella colonisation in the challenged birds.

4.3.1 Organ weights

Generally, the control birds had lower duodenum, jejenum, ileum, caeca and liver weights, than those birds that were challenged with Salmonella. As the liver and caeca are the main sites of Salmonella colonisation it would be expected that the control birds had the lowest weights while the Salmonella infected birds had the highest weight, which was shown to be the case. These findings are similar to those of Calnek *et al.* (1991), who found that ST infection resulted in enlarged liver, spleen or kidneys with consequently higher organ weights for these infected organs.

4.3.2 Broiler performance

4.3.2.1) Body weight and daily gain

Salmonella had an inhibitory effect on growth, especially during the first three weeks of life. This is confirmed by Du & Wang (2005) who also found a reduction in body weight gain for chicks infected with high doses of ST. Salmonella infected chicks from the non-antibiotic groups had higher body weights than those that received antibiotics. The inclusion of Zinc-Bac into the diet had no effect on the control birds and even went so far as to inhibit the growth of the birds exposed to ST. Zinc-Bac is included in the majority of poultry diets in South Africa, but in this trial proved to have very little positive effect as a “growth promoter”.

The inclusion of the Zinc-Bac would appear to have killed off or inhibited the potentially beneficial microflora in the birds’ intestinal tract, thereby reducing the competition for nutrients and adhesion space that the ST would otherwise have normally encountered. As it is only affective against Gram-positive species, the ST, which is Gram-negative, would appear to have been given a better chance to dominate in the intestinal environment, which is shown by the inhibitory effect that the ST had on BW of the birds.

The birds that were challenged with Salmonella had similar body weights as the birds that were not challenged, this was especially apparent during the later stages of production. This observation serves to confirm that body weight alone is not a reliable indicator of Salmonella infection. It would also appear that the challenged birds were able to compensate for the early negative effects on growth to end up with the same body weights as the non-challenged birds.

4.3.2.2) Feed intake (FI)

According to Duke (1993), the hypothalamic centres affect FI. High environmental temperatures; high dietary energy levels and high dietary protein levels all lead to a decrease in FI, while low ambient temperatures; moulting and egg production all increase FI. Klasing (1998) found that systemic infection begins with acute phase protein synthesis in the liver and is followed by several behavioural, hormonal and metabolic responses in the broiler e.g. feed intake would decline in birds infected with ST. The control chicks had significantly lower FI than the ST infected chicks shortly after inoculation.

4.3.2.3) Feed conversion ratio

According to Capitan-Vallvey *et al* (2002), Zinc-Bac is used to improve growth rates and feed conversion in poultry, pigs and cattle. The addition of zinc bacitracin to the diets of Salmonella-challenged birds caused an increase in FCR during the first weeks of growth. These findings are contrary to those found by Huyghebaert & de Groote (1997); Engberg *et al.* (2000) and Van Poucke *et al.* (2003) all of whom claim that enhancements in animal performance in terms of growth rate and feed conversion ratio could be achieved with the use of Zn-Bac, with feed conversion improving in broilers given Zn-Bac in their diets. Bacitracin is one of the most common antibiotics used in the world and is found in many South African poultry diets as an animal feed additive due to its growth-promoting effects. These “apparent” growth- promoting effects did not appear throughout this trial, with the Zn-BC rather having no effect on the growth and giving poor FCR in the birds that did receive the Zn-BC in their diets.

4.3.3 Serum biochemical profile

Plasma protein synthesis decreases in severe liver damage (Tang, 1975). According to Duke (1993), plasma makes up between 55-70% of the blood, and plasma protein synthesis decreases in severe liver damage. Plasma proteins consist of two major types: albumin and globulin. Albumin is the most abundant protein in the plasma, and is the major protein produced by the liver (Duke, 1993; Frandson & Spurgeon, 1992). Albumin is important in the binding and transporting of many substances in the blood and is responsible for about 80% of the total potential osmotic pressure (oncotic pressure) of the plasma as it is a high molecular weight protein that does not pass readily through the vessel or capillary walls it therefore aids in keeping fluid in the vascular system (Frandson & Spurgeon, 1992).

Globulin is a reactive protein and a plasma precursor with Gamma-globulins being stimulated by the presence of antigens and synthesised by the plasma cells (Frandson, & Spurgeon, 1992) and lymphocytes containing the antibodies known as immunoglobulins (Duke, 1993). Gamma-globulin is associated with immunity and resistance to disease. It is responsible for providing the immune response. The Gamma-globulin content of the blood therefore increases following vaccination and during recovery from disease. Beta-globulin transferin combines with and carries iron from the blood capillaries in the mucosa. The Alpha- and Beta-

globulins are synthesised in the liver. Globulin and albumin are simple proteins that yield only amino acids or their derivatives upon hydrolysis (Duke, 1993).

In this study, serum albumin levels of the birds that were challenged with ST were lower than in the control birds shortly after inoculation took place. From day 14, this effect could no longer be detected. This could be an indication of early liver damage with relatively quick recovery. Globulin levels were also significantly decreased at day 7 in broilers inoculated with the lower level of Salmonella. However, for the higher ST level at day 7, and both ST levels at day 14, ST exposure caused broilers to have increased levels of serum globulin. The initial decreased globulin levels could again indicated liver damage, but with the recovery of the liver, more globulin was being produced in reaction to the high levels of antigens (ST) present in the body. The increased levels of globulin could also have been a sign that the birds were recovering for the ST infection.

Total plasma proteins are a common endpoint utilised to estimate avian body condition (Rajman *et al*., 2006). Birds infected with ST showed significantly elevated antibody levels in the serum than those of the control birds (Lee *et al*., 1983; Beal *et al*., 2004; Du & Wang, 2005; Beal *et al*., 2006; Okamura *et al*., 2007) rising to a peak at day 29 of infection (Lee *et al*., 1983) which supports the findings of this trial.

Aspartate is catabolised to produce fumarate by way of the urea cycle. Birds excrete excess amino nitrogen as uric acid (Duke, 1993). Plasma AST activity may reflect changes in hepatic function and can be used as a biochemical indicator for hepatic damage (Adav & Govindwar, 1997), a marker enzyme for hepatocellular necrosis (Bintvihok & Kositcharoenkul, 2006; Coulombe *et al*., 2005; Frankič *et al*., 2006; Han *et al*., 2008; Rishi *et al*., 2006) and alterations in muscle membrane permeability (Brenes *et al.,* 2003; Rajman *et al.* 2006; Corduk, 2007). Corduk *et al.* (2007) stated that an increase in AST activity is an indicator of a progressive liver cell injury followed by an increased production of reactive oxygen species due to external factors such as heat, trauma, infection, toxin and exercises. According to Brenes *et al*. (2003) and Rajman *et al*. (2006), plasma AST is not so specific and sensitive to hepatocellular damage in birds as it is in mammals, but Denli *et al*. (2004) regarded the activity of AST in serum of broilers a sensitive indicator of acute hepatic necrosis.

For this trial, aspartate transaminase activity did not differ between treatments. However, there was a general trend for increased levels of AST during the first two weeks after Salmonella inoculation, which again might reflect liver injury during this phase.

4.3.4 Intestinal damage: lesions

According to Berndt & Methner (2001) and Berndt & Methner (2004), there were no signs of intestinal inflammation in birds that were orally administered a ST vaccine, although some

birds challenged with ST showed a slight inflammation of the intestine in the first week after infection. In trials done on mice infected with ST results included atrophy with ischemic necrosis in the small intestine mucous layers (Lee *et al*., 2006; Rishi *et al*., 2006).

Post mortem, findings can range from complete absence of visible lesions to a septicaemic carcass (Jordan & Pattison, 1996). Some birds may show signs of lesions identical to any acute septicaemia. Lesions may be absent in extremely severe outbreaks. Adult birds can show signs of necrotic ulcers in the intestines, although chronically infected adults frequently exhibit no lesions.

No lesions were found in any of the chicks slaughtered during the first two slaughters, while lesions did occur in the final three slaughters. There were no statistically significant differences for the number of chicks in which lesions occurred. In general, the control chicks had fewer numbers of birds with lesions than the ST infected chicks.

For severity of lesions, no significant differences were found between treatments for chickens slaughter at 21 days of age. The results for the birds slaughtered on days 28 and 35 of age showed that the inclusion of zinc bacitracin in the feed did not affect the severity of intestinal lesions. However, there was a significant difference in lesion scoring between the control birds and the Salmonella infected birds, with the control birds having lower lesion scores than the groups inoculated with Salmonella. No significant differences were found between the ST levels suggesting that the dosage of ST does not influence the severity of the lesions. Measurement of lesion severity could be used as an indicator of ST infection in further studies.

4.3.5 Histopathological results

The caeca were the only organs to consistently show signs of histopathology for all birds slaughtered, while histopathology for the liver and bursa were minimal and inconsistent. This is in contradiction with numerous findings in the literature. Allameh *et al*. (2005), Coulombe *et al*. (2005), Denli & Okan (2006) and Méndez-Albores *et al*. (2007) found extensive liver damage in trials conducted on chickens, with hepatocellular necrosis, multifocal fatty degeneration with large fat droplets displacing the nucleus resulting in fatty liver. There was leukocyte infiltration, congestion, hyperplasia of the epithelium as well as biliary hyperplasia. The livers appeared friable and yellowish in colouration. According to Calnek *et al.* (1991), young birds showed signs of congested liver and spleen with haemorrhagic streaks or necrotic foci, congested kidneys as well as pericarditis with adhesions, while adult birds showed signs of congested and swollen livers, spleen and kidneys with haemorrhagic or necrotic enteritis, pericarditis and peritonitis. This is supported by Jordan & Pattison (1996), who found signs of swollen lungs, liver, spleen and kidneys. Unabsorbed yolk sacs have been

seen in young chicks. Necrotic lesions in the lungs, liver and heart, peritonitis, typhlitis and haemorrhagic enteritis are all symptoms that can be seen with Salmonella infection.

In trials done on mice infected with ST results included atrophy with ischemic necrosis in the small intestine mucous layers as well as severe haemorrhagic necrosis within the pulp of the spleen. The liver was congested with polymorphonuclear leukocyte infiltration, fatty deposits, structural disintegration of hepatic plates, haemorrhage, necrosis and the presence of necrotic foci (Lee *et al*., 2006; Rishi *et al*., 2006).

4.3.6 Villous morphological measurements

No significant differences were found between the treatments for villi measurements taken from the birds slaughtered at day 14 of age. Villi measurements taken at day 28 of age showed some significant differences. There was a significant difference between antibiotic groups that were inoculated with 2 x 10^9 CFU ST/mL, with those birds receiving antibiotics having longer duodenal villi lengths than those that did not receive antibiotics. Dibner & Richards (2006) found that AGP effects that occur in germ-free animals include reduction in gut size, including thinner intestinal villi and total gut wall, while Mourão *et al* (2006) found that rabbits fed an AGP had significantly longer villi compared to the unsupplemented control. It was found that feeding an AGP significantly increased villi length in the ileum.

On day 28 of age, the duodenal and jejenal villi lengths of birds that were challenged with Salmonella, were shorter than in those birds that were not challenged. This trend was also noted for villi length in the ileum of birds challenged with ST that received zinc bacitracin in their feed. Edens *et al*. (1997) found that in birds challenged with Salmonella at hatch, the ileum villi became shortened and blunted, while the control birds' ileum villi retained their tall, cylindrical morphology. .

Short villi result in an impaired absorption for two reasons. First, shortening results in an absolute loss of intestinal surface area. Second, cells that are lost are generally the mature cells. Because nutrient absorption is necessary for osmotic water absorption, water absorption is decreased by impaired nutrient absorption (Montagne *et al*., 2003).

4.4 Conclusion

ST infection resulted in enlargement of the liver possibly from hepatic damage. This finding together with those of the serum biochemical measurements of decreased albumin levels which indicate early liver damage, increased globulin levels indicating recovery from disease, as well as increased TSP levels indicating severe liver damage, can be used in conjunction to measure the effect of ST on liver damage.

ST was shown to inhibit the growth and BW of infected birds, while no significant differences were found for the level of antibiotic used, thereby stating that the antibiotic used was providing no growth promoting effects at all. Zinc-Bac may in fact have inhibited potentially beneficial microflora allowing the ST to proliferate in the intestinal tract of infected birds.

The severity of intestinal lesions may be a good indicator of ST infection during the later stages of production.

While the control birds had fewer histopathological findings than those birds infected with Salmonella, the results were inconsistent with those found in the literature and can therefore not be used as a reliable indicator of Salmonella infection. The caeca was the only organ to show consistent results. It may be that higher levels of infection will be required to observe histopathological effects in the liver and bursa of infected birds.

The measurement of the intestinal villi proved to be a good indicator of infection in the later stages of production, with the control birds having longer villi than the birds infected with ST.

It would appear that higher levels of ST may be required in further studies in order to obtain early, consistent observable indications of infection. A combination of cloacal swabs, the only procedure that proved to be 100% effective in the indication of Salmonella infection, BW measurement, liver and serum measurements, intestinal lesion scoring, caecal histopathology and villi measurements all assessed in conjunction with one another would appear to be effective in the measurement of ST infection in broilers. The difficulty may come in the interpretation of the results, as on their own the symptoms displayed by the broilers could also be an indication of other disease infections.

In future trials higher levels of ST infection should be employed in an attempt to obtain more consistent and observable results. Maternal antibodies may still have played a role in this trial; therefore specific pathogen free chicks should be used in future to rule out this effect.

The alternative hypothesis that colonisation will have quantifiable effects therefore making evaluation of alternative products possible, has been proved. The null hypothesis that Salmonella colonisation of the gastrointestinal tract of broiler chicks does not have any quantifiable effects on the birds therefore making the evaluation of alternative products to control Salmonella infection impossible has been disproved.

Chapter 5
General discussion and conclusions

Biosecurity is one of the most important aspects to consider when doing a trial with Salmonella. Results from the weekly cloacal swabs taken throughout the trials showed that the biosecurity measures which were employed were effective in the prevention of cross-contamination between the Salmonella-challenged and the non-challenged birds. The cloacal swabs also showed that the use of zinc bacitracin as an antibiotic in the second trial did not inhibit Salmonella colonisation in the challenged birds. The cloacal swabs proved to be an effective yet time consuming tool for the determination of Salmonella infection. While the cloacal swabs are a useful indicator of Salmonella infection, it can not be used as the sole method of Salmonella detection as it gives no indication as to the severity of infection. According to Calnek *et al*. (1991), the reliability of cloacal swabs as a diagnostic tool for PT infection appears to be limited in that faecal excretion of the organisms may be intermittent and therefore not reliable. More detection and evaluation criteria need to be incorporated into future studies.

In both of the trials, Salmonella challenge resulted in enlargement of the organs and therefore an increase in the organ weights. Salmonella-challenged birds showed a reduction in body weight, average daily gain and feed intake with an increase in the feed conversion ratio. Results from the first trial showed a reduction in all serum levels in Salmonella-challenged birds, while the non-challenged birds that received cyclophosphamide had elevated serum levels. Results from the second trial showed that Salmonella-challenged birds had reduced albumin levels but elevated globulin, TSP and AST levels.

ST infection resulted in enlargement of the liver possibly from hepatic damage. This finding, together with those of the serum biochemical measurements of decreased albumin levels which indicate early liver damage, increased globulin levels indicating recovery from disease, as well as increased TSP levels indicating severe liver damage, can be used in conjunction to measure the effect of ST on liver damage.

Salmonella challenge resulted in higher lesion numbers as well as increased severity of lesions seen in the gastrointestinal tract. Histopathology results proved to be inconsistent and did not provide any conclusive evidence on the effect of Salmonella on the organs. Villi measurements taken in the second trial showed that Salmonella shortened the length of the villi in challenged birds.

Although the first trial conducted showed that the cyclophosphamide was not necessary to suppress the immune system in order to infect the birds with Salmonella, it may be prudent to

use cyclophosphamide in further studies in order to yield statistically significant and reliable results.

Bacitracin is one of the most common antibiotics used in the world as an animal feed additive due to its growth-promoting effects and Zinc-Bac is included in the majority of poultry diets in South Africa. In this trial the Zinc-Bac showed no growth promoting benefits. Instead, the inclusion of the Zinc-Bac appeared to have inhibited the potentially beneficial microflora in the birds' intestinal tract, thereby reducing the competition for nutrients and adhesion space that the ST would otherwise have normally encountered. This allowed the Salmonella to proliferate in the body of the chicken, which lead to the conclusion that the routine inclusion of ZN-Bac at sub-clinical levels as a growth promoter may be detrimental when the bird gets exposed to Gram(-) bacteria, such as Salmonella.

Perhaps limiting the second trial to only three levels of Salmonella infection was a mistake, and in any future studies more levels should be incorporated. Ideally, the number of birds used in both the trials should have been larger, and the number of birds slaughtered at each week should have been increased to prevent large variation in the results. The lesion scoring and villi measurements could prove to be effective tools during the later stages of production.

It would appear that using all of the information and results obtained for organ weights, broiler performance, serum biochemical level, lesion scoring, histopathology and villous morphological measurements should be used in conjunction with one another to measure the effect of Salmonella on the broiler chicken.

Chapter 6
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Appendix

Millonig's Buffered Formalin solution

To prepare 50ℓ of a 10%- buffered Formalin solution (which prevents shrinking of tissue)

- 857,2 g NaH_2PO_4
- 173,9 g NaOH
- 242,8 g $C_6H_{12}0_6$ (glucose)
- 5l H_2CO 40%-Formalin (methanal)

Method:

- Dissolve each of the dry chemicals, separately in 1800ml H_2O on a magnetic stirrer.
- Add the 3 solutions to the 5ℓ formalin in a 50ℓ container.
- Fill up to 50ℓ with TAP water.

Rambach Agar

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25ºC. Protect from light. After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25ºC.

Method:

- Add 1 vial of liquid-mix to 250, 1000 or 50.000 ml distilled water and mix by swirling until completely dissolved (the water quantity is dependent on the respective pack-size).
- Add 1 vial of nutrient-powder and mix by swirling until completely suspended.
- Heat in a boiling water-bath or in a current of steam, while carefully shaking from time to time. The medium is totally suspended, if no visual particles stick to the glass-wall.
- The medium should not be heat-treated further!
- Standard time for complete dissolution (shaking in 5-minute sequence):
- 250ml: 20-25 minutes
- 1000ml: 35-40 minutes.
- Do not autoclave, do not overheat!
- Cool the medium as fast as possible in a water-bath (45-50ºC). During this procedure (max. 30 minutes) gently shake the medium from time to time. Pour into plates.
- In order to prevent any precipitate or clotting of the chromogenic-mix in the plates, we advise to place Petri dishes –during pouring procedure- on a cool (max. 25ºC) surface.

- The ready-plates are opaque and pink. Before inoculation, the plates should be dry. pH 7.3 ± 0.2 at 25ºC.
- Shelf-life and storage conditions of fresh prepared plates: room-temperature: 12 hours
- In the fridge (not below 6ºC) unsealed: 3 weeks
- In the fridge (not below 6ºC) sealed in plastic pouch or with tape: 3 months.

References

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Rappaport-Vassiliadis broth

Preparation

Suspend 41.8 g/litre, heat gently, if necessary dispense into test tubes, autoclave gently (15 minutes at 115ºC).

pH 5.2 ± 0.2 at 25ºC.

The broth is clear and dark-blue.

The prepared culture medium can be stored in the refrigerator for at least 7 months.

References

Merck Microbiology Manual 12th Edition.