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System dynamics model of necrotic enteritis and its predisposing factors in broilers

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Necrotic enteritis (NE) caused by *Clostridium perfringen*s type A is an important bacterial enteric disease of global broiler production. However, the dynamic interactions of NE and its predisposing factors are not fully presented by current studies. By using the System Dynamics (SD) Model, the epidemiological changes in susceptible-infected-removed models of NE and avian coccidiosis and their interactions in one or multiple grow-out cycles was established; meanwhile, the growth performance was measured by the average weights of infected and non-infected populations at harvest were estimated. The SD model provided direct and persuasive outcomes of the epidemiology and ecology of NE compared with models using statistical methodology. With interventions on certain predisposing factors of management practices and medication, effects which decreased disease incidence and growth performance were observed; moreover, the leverage points obtained from interventions on certain management practices provided quantitative results which were applicable and useful for improving the broiler production.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

LITERATURE REVIEW

1.1 NECROTIC ENTERITIS

1.1.1 Etiology

Necrotic enteritis (NE) is a significant bacterial enteritis caused by *Clostridium perfringens*, which affects the global poultry industry and has been estimated to cost up to US$6 million per year in production losses and measures of mitigation and prevention (Wade and Keyburn, 2016). It is also associated with other significant poultry diseases, including gizzard erosion and ulceration syndrome (Fossum et al., 1988) and gangrenous dermatitis (Weymouth et al., 1963). *Cl. perfringens* is a gram-positive, anaerobic, spore-forming bacterium that is transmitted by the fecal-oral route (Wise and Siragusa, 2005) and widely exist in the integrated broiler production (Craven et al., 2003) as well as in the environment ,such as soil and water (Cox et al., 2005; Desmarais et al., 2002). There are 5 toxigenic types, A to E, of *Cl. perfringens* producing four major toxins (alpha, beta, epsilon and iota) that cause enteric and cholangiohepatic infection (Cooper and Songer, 2009) at the age of 2-5 weeks (Ficken, 1991). In recent years, the primary causative agent of NE in broiler chicken was identified as *Cl. perfringens* type A strains carrying the NetB gene and dominantly expressing the pathogenic toxin (Keyburn et al., 2008, 2010) which induce enterotoxemia in the intestinal tract. Normally, Clostridia live commensally in intestine tract of chickens (Timms, 1968) and form the major part of the cecal flora

with other anaerobic bacteria (Barnes et al., 1972). In Barnes' study, low number of Clostridia and *Cl. perfringens* was identified in the duodenum and small intestine of 2 to 6 week-old chickens fed with 9% fish meal. It was identified that contaminating hatchers and feed might be the sources of *Cl. perfringens* or other Clostridia that gradually influenced the establishment of intestinal flora from 0 to 3 day-old chicks (Barnes et al., 1980) or even to 2 weeks of age (Shane et al., 1984).

The pathogenic strain, also called enterotoxigenic *Cl. perfringens*, had an incidence of 10% in poultry fecal samples (Tschirdewahn et al., 1991). The interaction and competition between pathogenic *Cl. perfringens* strains and other microbiota in the gut affect induction and severity of NE (Antonissen et al., 2016). A study of artificial boosting of *Cl. perfringens* in chickens demonstrated that the member of Clostridiales were present in similar abundance between infected and control birds with a shift in population towards *Cl. perfringens* at the expense of other family members (Stanley et al., 2012). In studies investigating the strains of *Cl. perfringens* in chickens in Sweden and Denmark, the results of polymerase chain reaction (PCR) showed that all 279 isolates were *Cl. perfringens* type A, and pulsed-field gel electrophoresis (PFGE) demonstrated that healthy chickens carried several different clones within the flocks or individual birds, while NE infected birds carried only one or two clones (Engström et al., 2003; Nauerby et al., 2003). Therefore, under some circumstances, the pathogenic strains of *Cl. perfringens* become more prominent in response to the predisposing factors which play critical roles both in disease outbreaks in the field and in models of experimental induction (Stanley et al., 2014).

1.1.1.1 Clinical NE

The occurrence of clinical NE is caused by swiftly increasing proliferation of pathogenic *Cl. perfringens* that continuously produces the extracellular toxins damaging the small intestine (Keyburn et al., 2006). The clinical signs of NE infected chickens include marked depression, reluctance to move, diarrhea and ruffled feather. In gross pathology, chickens dying from NE had enlarged intestine with gas and brown fluid content. The necrotic lesions could be observed from jejunum to ileum of small intestine where the damaged mucosa was devoid of villi tips covered by a tightly adherent diphtheritic membrane (Bains, 1968; Helmboldt and Bryant, 1971), but could occur in duodenum and ceca (Van Immerseel et al., 2004). There are several lesion scoring systems used to measure the severity of NE which have ranges from 0 to 5scores (Brennan et al., 2003) or 0 to 3 scores (Hamdy et al., 1983). The scale of 0 to 5 scores is most commonly used in field or experimental research (Cooper and Songer, 2009). In this scale, ill chickens with an increasing severity of visual lesions starting from the thin-walled, friable intestine to extensive necrosis in small intestine will be scored from 1 to 4. Birds often die from NE with a lesion score that was more than 4. The classical acute clinical form of the disease is characterized by a sudden increase in flock mortality often without premonitory signs, and the course is often per-acute with death in 1 to 2 hours. The subclinical form of NE shows no overt clinical signs and no peak mortality; however, the chronic intestinal mucosal damages cause production losses because of the reduction of weight gain as well as higher feed conversion ratio (Timbermont et al., 2011). Through the increasing prevalence and barely detectable occurrence of subclinical

form, the poultry industry has been aware of its consequence which leads to greater economic losses than the clinical NE.

1.1.1.2 Subclinical NE

The mild form of NE, known as subclinical NE (SNE), is one form of dysbacteriosis caused by an imbalance of the normal microbial flora in the small intestine and initiated by a mixture of opportunistic pathogens, such as *Cl. perfringens* (Kaldhusdal and Hofshagen, 1992; Palliyeguru and Rose, 2014). The subclinical NE is most likely to be detected in chickens at the age of 3 weeks in several experimental models (Kaldhusdal and Hofshagen, 1992; Lovland and Kaldhusdal, 2001; Wu et al., 2010). It causes superficial focal ulcerations in the range of 1-5 mm in diameter at the part of apical villi accompanied by impaired growth performance with or without clinical signs (Brennan et al., 2001b, 2001a; Kaldhusdal et al., 1999; Shane et al., 1984). Due to the focal ulceration that resulted in fluid loss and nutrient malabsorption, the mortality in affected flocks was low, but growth rate was reduced (Wilson et al., 2005). This would lead to the decreasing weight gain and feed intake, and clinical depression could often be observed. From little infiltration of inflammatory cells to multifocal hepatic necrosis, these hepatic lesions , also called *Cl. perfringens* associated hepatitis (CPH), were described in infected chickens with clinical and subclinical form of NE at processing (Hutchison and Riddell, 1990; Løvland and Kaldhusdal, 1999; Onderka et al., 1990). The transportation of the bacteria, bacterial toxins and damaged tissue products to liver via the portal blood might be the causative pathway (Lovland and Kaldhusdal, 2001; Onderka et al., 1990; Sasaki et al., 2000). It was demonstrated that, during subclinical infection, bacteria can reach the portal blood stream and bile duct, and colonization of high

numbers of *Cl. perfringens* in hepatic tissue resulted in cholangiohepatitis; however, during meat inspection at processing plants, condemned livers were found, without any clinical signs in the flock (Timbermont et al., 2011). The variation in severity of the necrotic enteritis has been induced in the different models or observed in field, such as very low levels of gut necrosis in only a minority of treated animals which was suitable for studying the subclinical NE (Gholamiandehkordi et al., 2007).

1.1.2 Epidemiology

Necrotic enteritis was first reported and considered as an enterotoxaemia due to *Clostridium Welchii* (Ma et al., 2012), in England in 1961(Parish, 1961); after then, it had been subsequently described from most poultry producing areas, including the U.S., Canada, and Australia (Bains, 1968; Helmboldt and Bryant, 1971; Long, 1973; Nairn and Bamford, 1967). The outbreak in England occurred in 110 cockerels of 6 to 7 weeks of age with 38 birds that died after 4 days from the onset of the acute form. Most of the chickens that died from the acute form had lesions of necrosis in the small intestine containing fluid with the diphtheritic membrane and congestive livers caused by toxemia. In Western Australia, NE occurred on at least 30 different poultry farms and mainly affected chickens of 2 week-old to 7 week-old from September to December in 1963. The short period of illness, no observation of clinical diarrhea and dead birds were usually found with the mortality rarely exceeded 5 % (Nairn and Bamford, 1967). A similar condition that occurred in north-east Australia was briefly described with a high mortality of affected broiler chickens of 5week-old that had clinical responses after 72 hours of antibiotics treatment (Bains, 1968). The course of 75 outbreaks occurred in Maine and Connecticut in 1969 was approximately 7 days with a daily mortality rarely exceeding 1

%; however, the morbidity was difficult to be determined visually because the infected birds died rapidly (Helmboldt and Bryant, 1971). The thorough investigation in Canada demonstrated the several similar results compared to previous reports, such as daily mortality rose suddenly with no morbidity data available, but showed the reproduction of the disease which provided significant findings (Long and Truscott, 1976). In their study, it was found deaths occurred within 24 hours after taking inoculated feed; however, deaths were more commonly found after 36 hours with the peak mortality which reached at 48 hours. The acute nature of NE was often seen with sick birds dying within 30 minutes after illness was observed. With the exposure to *Cl. perfringens* for 24 hours feeding period resulted in 12% mortality while a 5 day feeding period resulted in a maximal mortality of 26%. This finding suggested a relationship between continued exposure to the pathogens and incidence; furthermore, since a maximum of mortality was also observed, it was apparent there were other factors important in reproducing NE (Long and Truscott, 1976). In an early experimental model, by daily feeding mixed ration of *Cl. perfringens* and *Eimeria acervulina*, the mortality on the 5^{th} through 7^{th} days after infection was considered to be consistent with the hypothesis that mucosal lesions and physiological changes, induced by coccidiosis, were responsible for the onset of NE (Shane et al., 1985). In this study, the author mentioned that the morbidity and mortality ranging from 5-10% and 0.5-1%, respectively, could be the result of consecutive outbreaks on a farm; besides, the result demonstrated that the concurrent and prior infection with the sexual stage of *E. acervulina* exacerbated the mortality with the incidence of 35% and 41% owing to the damage of intestinal mucosa.

1.1.2.1 Occurrence of *Cl. perfringens*

Regarding the occurrence of *Cl. perfringens*, the early research studies indicated that it could exist in the integrated broiler operation. By detecting the pathogen in fecal or cecal samples, 15 (94%) of the 16 flocks in a year had positive result of *Cl. perfringens* and only one flock remained negative throughout the 6 to 8 week rearing period (Craven et al., 2001). This study also demonstrated that 13 of the flocks were *Cl. perfringens* positive at 2 week of age when sampled biweekly through grow-out. Of the on-farm environmental samples, the highest positive percentage *of Cl. perfringens* were detected in wall swabs (53%) and the lowest incidence was detected in swabs of workers' boots (29%). In the procession plant, *Cl. perfringens* was recovered from broiler carcasses after chilling in 13 (81%) of the 16 flocks. The proportion of *Cl. perfringens*-positive carcasses for the contaminated flocks ranged from 8% to 68%. A later study also demonstrated a similar pattern by detecting the ribosome types of certain strains of *Cl. perfringens* from the samples collected from the breeder farms, hatchery, previous grow-out flock and the processing plant. The higher positive percentage of *Cl. perfringens*-positive breeders had higher percentage of *Cl. perfringens*-positive samples in hatchery and broiler farms. When a higher percentage of *Cl. perfringens*-positive samples was detected in a grow-out flock, the next flock at 3 weeks of age had a higher positive percentage (Craven et al., 2003). By using the nested polymerase chain reaction (nested PCR) and most probable number (MPN) method, the positive percentage of enterotoxigenic (pathogenic) *Cl. perfringens* in intestinal contents and meat of slaughtered broiler chickens were 40% ($4.0x10^2$ - $9.3x10^7$ MPN/100g) and 12% ($<10^2$ - $4.3x10^2$ MPN/100g) of 50 birds. In comparison with the positive percentages of enterotoxigenic (pathogenic) *Cl. perfringens*,

the positive percentages of total *Cl. perfringens* were 80% ($4.3x10^2 – 9.3x10^7$ MPN/100g) and 84% ($<10^2 – 9.3x10^3$ MPN/100g) (Miwa et al., 1998, 1997).

The prevalence of NE was estimated as 7.7 % by broiler samples diagnosed in 3 laboratories in Canada (Long, 1973). These samples diagnosed as NE involved some outbreaks, but more than one consignment was submitted. Another study showed two peak prevalence of 34.8% and 25.3% during the years 1969-1989 in Norway by collecting consignments diagnosed as NE in the central veterinary laboratory and estimating the numbers of broiler flocks in the population based on total numbers of birds and estimates of average flock size (Kaldhusdal and Skjerve, 1996). The study also indicated that NE occurred more often in winter than the late spring to summer time. Outbreaks of NE are sporadic, and it can occur more than once a year on a particular farm (Tamirat et al., 2017). According to a survey study in the United Kingdom (UK), a farm-manager-reported point prevalence of NE was 12.3% based on the information collected from 72 % of the UK commercial broiler population in 2001 and 2002 (Hermans and Morgan, 2007). NE occurred at a median age of 26 days and was reported as early as 10 days of age and as late as 49 days. The multivariable analysis of the study demonstrated the occurrence of NE had a strong association with farmer-observed coccidiosis and wet litter. The plaster-board walls used by 6% of surveyed farms were also found as a risk factor which indicated that hygiene and disinfection had an important part of the prevention of NE. The use of ammonia as a disinfectant, especially for coccidial oocysts, in broiler houses at the last flock applied by 8.6 % surveyed farms had no significant association with the occurrence of NE. Preventive measures, such as the prophylactic use of antibiotics and competitive exclusion products, were not found to be significant factors

in the model; however, at least one prescription-only antibiotic for preventive reasons in the most recently reared flock had been used by 51% of farm managers. Because of leaving out independent growers and smaller companies, the selection bias could exist in the study. In addition, the positive association between the farm size and NE occurrence might be overestimated due to the non-responding farms having fewer chickens on average. Unfortunately, published studies regarding NE occurrence are few and make it difficult to achieve accurate estimates (Kaldhusdal et al., 2016).

The diagnosis of subclinical NE is difficult because it may be suspected from unapparent clinical signs, such as sticky droppings, wet litter conditions, suppressed growth and reduced feed conversion efficiency (Palliyeguru and Rose, 2014). Consequently, the morbidity of subclinical form is difficult to determine in field but could be estimated by the gut lesion scores and CPH in some experimental models. In a study of detecting the serum antibodies to α-toxin of *Cl. perfringens* in farms of Norway, the single flock with clinical NE sampled was serologically positive. In two experimental groups where the occurrence of induced SNE had been high (unpublished data), a considerable proportion (59 and 79%) of sampled birds were seropositive (Lovland et al., 2003). In another study of quantifying the gut lesions in subclinical NE model, 43% (30% to 62%) the flock groups co-infected with *Cl. perfringens* and coccidia developed macroscopic gut lesions in average, and the time interval during which lesions were detected was from day 16 to 26 (Gholamiandehkordi et al., 2007). Information regarding subclinical NE is comparatively rare with the clinical form; therefore, a scoring system needs to be validated by investigation of a larger number of birds (Pedersen et al., 2008).

Although clinical forms of NE can cause high mortality, the subclinical form cause huge economic loses in broiler production because it persists in the flock without any clinical manifestation and treatments (Dahiya et al., 2006). Predisposing factors exist in the external and internal environment of birds and cause conformational changes to the gut, influence of immune status and disruption of microbiota (Moore, 2016). Overall effects presented by these predisposing factors facilitate the incidence of NE, clinical or subclinical form, and adversely affect the performance of flocks, especially the subclinical form. The gradual reduction of feed intake and daily growth are noticed in chickens infected with subclinical NE which cause higher feed conversion ratio (FCR) and the economic loss can be as high as US$0.05/bird or higher (Van der Sluis, 2000a, 2000b).

1.2 PREDISPOSING FACTORS OF NE

1.2.1 Avian coccidiosis

1.2.1.1 Etiology and epidemiology

Among these predisposing factors, the incidence of coccidiosis caused by *Eimeria* spp. is a major factor facilitating the occurrence of NE. The *Eimeriidae* are homoxenous parasites (direct life cycle) which intracellularly undergo several cycles of the asexual reproduction (schizogony) followed by the last cycle of the sexual reproduction (gametogeny) in intestine and then develop into oocysts within the same host. The unsporulated oocysts are shed with the feces; once outside the host, the oocyst must sporulate before it is ingested and infective to another host animal. The prepatent period, from the time of infection to the appearance of the first oocysts in the feces, is often completed in a period of 7 days; however, the sporulation takes in a period of 2 days at

ordinary temperatures (Levine, 1961). Following ingestion by avian hosts, there are two phases of excystation that ensure sporozoites escape from cysts. The sporulated oocysts are broken in the gizzard firstly and then release inside sporozoites in the small intestine (Williams, 1995). Maximal counts of oocysts ranges from 6 to 9 days after infection (Allen and Fetterer, 2002). The patent period is the time period beyond the prepatent period during which unsporulated oocysts are detectable in the feces of the host without reinfection (Williams, 1995). Despite the age of the host, a study demonstrated that unsporulated oocysts of *E. tenella* were more resistant to the grinding action of the gizzard, whereas sporulated oocysts could release numbers of sporozoites (Williams, 1995).

Avian coccidia of genus *Eimeria* are host-specific and site-specific. Because there is no cross-species immunity, it is possible to have concurrent outbreaks of different *Eimeria* spp. in one flock ((McDougald and Long, 2003). In addition to the size and shape of oocysts, the site specificity and pathogenicity are also distinctive diagnostic characters of avian coccidiosis (Table 1.1). Infections can be seen in all age groups; meanwhile, clinical signs range from decreased growth to a high percentage of sick animals with diarrhea and high mortality. Chickens of severe infections recover in 10 to 14 days but slowly return to normal production. It is extremely rare for commercial chicken flocks to be free from *Eimeria* spp. (Williams, 1999). Coccidial infections can be classified in one of three ways (Williams, 2002): (1) Clinical coccidiosis, characterized by mortality, morbidity, diarrhea or bloody feces; (2) Subclinical coccidiosis, defined by gradually causing reductions in weight gain and feed conversion efficiency of the host without obvious signs; (3) Coccidiasis (Levine, 1961), a mild infection causing no

adverse effects on the host. Each species has its own morbidity, mortality and specific gross lesions (Table 1.2).

There are two stages, tissue cysts and oocysts being the keys to the epidemiology of coccidiosis. The oocyst is the only exogenous stage in life cycle and is found in every species. The four general determinants that lead the oocyst stage to affect the epidemiology of coccidiosis include: (1) Factors affecting the number of oocysts produced; such as the inherent reproductive potential, the pathogenicity of schizonts, stress, nutrition, strain of host, resistance, coccidial drugs etc.; (2) Conditions affecting the sporulation of oocysts, such as three factors known to affect sporulation--temperature, moisture, and aerobic microbes; (3) Factors effecting oocyst survival time and infectivity, such as temperature, moisture and ammonia concentration; (4) The physical and biological dispersal of oocysts, such as transmission by insects (Fayer, 1980). Day-old chicks are susceptible to coccidiosis but may develop minimal infections because oocysts are excysted more rapidly in chicks aged 4-6 weeks than those aged 0-3 weeks (Rose, 1967). Moreover, unexposed adult birds remain highly susceptible to all species. Outbreaks commonly occur at 3- 6 weeks of age, but seldom occur at less than 11 days (McDougald and Long, 2003). The degree of immunity acquired prior to developing the clinical signs may influence the severity of infection in flocks (Permin and Hansen, 1998). Because of species diversity and combination infections, it is difficult to estimate the global prevalence of coccidiosis in poultry. Moreover, they have different geographical distributions dependent upon climate, humidity, and host species (Godwin and Morgan, 2015), so that relevant investigation of their prevalence may be limited to certain regions or countries. Available data has been variables because the methodologies

used due to sample collection, agent identification and data analyses have been different for variable research objectives (Table 1.3).

1.2.1.2 The relationship of NE and avian coccidiosis

The development of a coccidial infection that damages the epithelium provides a suitable environment for clostridia to establish, multiply, and produce the toxins that cause NE. Under field conditions, coccidiosis can play an important role in the occurrence and severity of outbreaks of NE (Al-Sheikhly and Al-Saieg, 1980). It was also identified that birds infected with both coccidia and *Cl. perfringens* had more severe necrotic lesions and higher mortality than birds infected with *Cl. perfringens* alone. Normally, the mean *Cl. perfringens* counts in the contents of the lower intestine were higher than those of the upper intestine, especially in ceca. In a study of experimental concurrent infection with NE and *E. necatrix*, in co-infected chickens, the mean *Cl. perfringens* counts were significantly higher in the small intestine at all levels at 3 days than those of the uninfected controls. In chickens of the coccidium-alone-inoculated group, *Cl. perfringens* counts in the duodenum and jejunum were significantly higher than those of the uninfected controls; meanwhile, the increase of *Cl. perfringens* counts was especially evident 7 days after *E. necatrix* inoculation (Baba et al., 1997). In this study, concurrent infections resulted in higher mortality; meanwhile, the results evaluated by the lesion scores had more significant findings than by mortality.

Coccidiosis not only causes physical damage to the gut, but also synergistically induces mucogenesis through the induction of local T cell-mediated inflammatory responses as well as affect the growth of pathogenic microbes in the intestinal tract (Collier et al., 2008). It was elucidated that the mucin gene expression was elevated

above the non-infected baseline in all infected birds at day 20 and further increased at day 22 in *Cl. perfringens* -infected birds (without the ionophore, narasin). In the other way, as the extensive damage to intestine mucosa, the lumen becomes rich with plasma proteins, which contains amino acids, growth factors, and vitamins serving as substrate for clostridia growth (Van Immerseel et al., 2004; Williams, 2005). Consequently, the digestibility of nutrients of intestine is decreased which may increase nutrient availability for *Cl. perfringens*. It was identified that pre-exposure to *E. maxima* repressed the inflammatory cytokine responses provoked by *Cl. perfringens*; therefore, the exacerbated lesions and increased *Cl. perfringens* colonization were found in the concurrent infection (Park et al., 2008).

Anticoccidial drugs which have been available since the 1940s provide the protection and treatment in chickens (McDougald et al., 1972); meanwhile, because clostridia are sensitive to certain ionophores (Liu, 1982), which are for in-feed prophylactic use, these ionophores may be used to protect chickens against clostridioses with Gram-positive antibacterial activity (Williams, 2005). However, because of the resistance of coccidia to these anticoccidial drugs, the occurrence of clinical coccidiosis as a contributing factor was promoted and the protection against NE was reduced. Moreover, when anticoccidial vaccines are administrated to chickens, these drugs are not used at the same time because they may kill the coccidia in vaccines (Williams, 2002). Therefore, some considerations brought out the controversy of using drugs against NE and coccidiosis. In-feed antibiotic growth promoters (AGPs), which were banned in the EU and the US, and ionophores can provide protection against both organisms; however, the resistance generated by improper administrations may increase the occurrence of

diseases. As clinical coccidiosis predisposes chickens to NE, live vaccines which aim to induce mild coccidial infection may lead to an increasing risk of NE.

Some intriguing observations regarding the natural relationship between NE and coccidia has also been discussed (Williams, 2005). Firstly, NE is unlikely to predispose chickens to coccidiosis, since the enterocytes are destroyed and diphtheritic membrane impedes intraluminal dissemination of extracellular coccidial stages. Secondly, in studies of spontaneous NE, clostridia always occurred in the lesions but the presence of coccidia in tissues or feces was often inconsistent in the pathological findings. Thirdly, heavily concurrent infection produced more severe pathogenic effects than either infection alone with *E. acervulina* (Al-Sheikhly and Al-Saieg, 1980)*, E. maxima* (Williams et al., 2003) or *E. necatrix* (Baba et al., 1997); whereras, small inocula of either organism or the random exposure to litter with oocysts had often failed to reproduce NE. Fourthly, clinical coccidiosis is not necessarily followed by NE unless sufficient numbers of *Cl. perfringens* are present; moreover, NE can result from other predisposing factors in the absence of coccidiosis.

1.2.2 Wet litter

1.2.2.1 The definition and risk factors of occurrence

Another key factor, which connects external and internal influences on the broilers, identified as a host, is the litter. Poultry litter is a combination of accumulated chicken manure, feathers and bedding materials, which is typically wood shavings, sawdust, wheat straw, peanut hulls or rice hulls (Edwards and Daniel, 1992). Thus, depending on multifactorial effects, such as types of bedding (Fasina, 2006), water intake (Collett, 2012), feed ingredients (van der Hoeven-Hangoor et al., 2014), house ventilation

(Weaver and Meijerhof, 1991) etc., the range of litter moisture content varies. It was found that poultry litter typically had moisture content of 18–21% (wet basis) (Fasina, 2006); otherwise, some litter moisture contents were reported as 19 to 31% (Chamblee and Todd, 2002), 30 to 33.5% (J. L. Glancey and S. C. Hoffman, 1996), 22.7 to 25.5% (Miles et al., 2006), 22.6 to 36.4% (Miles et al., 2008), and 25.6 to 29.7% (Sistani et al., 2003). In addition to absorbing moisture, litter needs the ability to release moisture which may decline as it becomes wet; therefore, its moisture content varies temporally and spatially during each grow-out period. The inherent capacity of holding water in litter materials determines when the litter reaches the critical moisture content and is defined as wet litter (Dunlop et al., 2016b). Wet litter can be compressed easily because of its reduced friability (Bernhart et al., 2010; Bernhart and Fasina, 2009) and is prone to form the manure cake on the surface of litter which increases the issues associated with the wet litter, such as bird health (Collett, 2012; Hermans et al., 2006) and ammonia concentrations (Miles et al., 2011a). Essential properties for all bedding materials to reduce the incidence of wet litter include good water holding capacity, reasonable drying rates (Grimes et al., 2002; Tucker and Walker, 1992), litter friability, susceptibility to cake formation and water activity (Garcês et al., 2013).

A positive correlation between poor litter condition and sticky droppings was identified (Elwinger and Teglof 1991). Diarrhea may be associated with acute NE (Helmboldt and Bryant, 1971), but not always (Nairn and Bamford, 1967), although the water to food intake ratio may be increased under this situation (Van der Sluis, 2000a). Several factors have been identified for contributing to the occurrence of wet litter (Table 1.4) (Dunlop et al., 2016b). In field surveys of broiler production conducted globally

(Van der Sluis, 2000a) and in the United Kingdom, the occurrence of NE was associated with diarrhea or wet litter; inversely, the occurrence of wet litter was associated with farms using side ventilation systems and winter time (Hermans and Morgan, 2007). The consistent risk factors determined by the multivariable analysis with two definitions, which were all cases of wet litter and cases of wet litter associated with disease, were clinical coccidiosis, breakdowns of feed equipment and the availability of separate clothing for each house (Hermans et al., 2006). A recent study in Australia, which surveyed experts of relevant disciplines, indicated that management of drinkers and house ventilation were the top two factors contributing to the wet litter within environmental or housing factors. Amongst the relevant diseases, coccidiosis and NE, were the mostly cited concerns (Dunlop et al., 2016b). It is difficult to find meaningful and specific solutions for housing which improve wet litter well because their designs are different based on different farms. It was suggested that house design and ventilation should improve to keep pace with genetics and nutrition which have substantially increased water excretion by birds over recent years (Collett, 2012).

1.2.2.2 The measurement of litter moisture content

The occurrence of wet litter can be contributed from the volume of water added to, evaporated from and stored in litter. The main sources of a large quantity of water absorbed in litter come from excretion and drinking spillage of high water intake and commercial stock density (Dunlop et al., 2016b). Among the properties measured with litter and bedding materials, moisture content (mass of water divided by mass of moist litter, expressed as a percentage) is commonly used, but caution is required when comparing the water holding capacity of different bedding materials because of the

differences in bulk density (Miles et al., 2011b). In Dunlop's study regarding the water addition, evaporation and water holding capacity of litter, it was found that evaporation rate increased with litter moisture content and air speed; besides, on a daily basis, evaporation rate of dry litter might not be sufficient to remove the volume of water added in the litter (Dunlop et al., 2015). Water activity (A_w) is an important thermodynamic property relating to the relative freedom or availability of water (Reid, 2007). It is a ratio of the fugacity of water in a system, and the fugacity of pure liquid water at a given temperature. The fugacity is a measure of tendency for a substance to escape. Generally, A_w is known to be related to microbial chemical and physical properties of natural products (Chirife and Fontana, 2007); nevertheless, it may be a better measurement of litter quality (van der Hoeven-Hangoor et al., 2014). Keeping the litter below the limit for microbial growth may control their proliferation, nominally: 0.86–0.90 for *Staphylococcus* spp., 0.92–0.95 for *Salmonella* spp., 0.95 for *Escherichia coli*, 0.9–0.97 for *Clostridium* spp., 0.98 for *Campylobacter* spp. and 0.75–0.85 for *Aspergillus* spp. (Fontana, 2007; Taoukis and Richardson, 2007). A_w increases non-linearly with litter moisture content. It was reported that litter A_w increased from 0.25 to 0.90 as moisture content increased from 10 to 31% (Bernhart and Fasina, 2009), and A_w increased to 0.98–0.99 when litter moisture content reached 38–55% (Carr et al., 1995). A_w gradients between litter and excreta control the flow of water; therefore, there is a necessity to maintain the litter moisture content below 30-35% and keep A_w of litter lower than A_w of excreta (Dunlop et al., 2016b). It was found that the increasing amount of excreta during the grow-out period caused the breakdown of the organic property of the bedding materials. This is because the higher speed of water evaporation from excreta into the air

which drew the water from bedding materials to excreta decreased the A_w of bedding materials. Thus, it was suggested that reused litter as bedding materials might provide some benefits given by its friability and lower A_w which could draw water from poultry excreta or air of houses (Dunlop et al., 2016a).

1.2.3 Feed changes

1.2.3.1 High-protein diets

Cl. perfringens requires 11 amino acids and almost all the growth factors and vitamins for its minimal growth (Doyle, 1989). Various hydrolytic enzymes produced by *Cl. perfringens* can act as additional virulence factors by facilitating the degradation of lysed cell substrates and providing nutrients for its growth (Petit et al., 1999). Therefore, the presence of high crude protein concentration and some required amino acids, especially glycine, may lead to an overgrowth of *Cl. perfringens* and production of bacterial toxins, such as alpha and NetB toxins, triggered by specific genes (Dahiya et al., 2005; Drew et al., 2004; Shojadoost et al., 2012). The level of *Cl. perfringens* has been found highest with the greater amount of animal protein (40% crude protein/feed) (Olkowski et al., 2006) and lowest in plant-source protein diets feed (Drew et al., 2004). It was found that dietary fish meal that has high glycine content was associated with *Cl. perfringens* proliferation and severity of NE (Kocher A, 2003). In some experimental models, changing the diet with soybean meal to a fish meal as a protein source before *Cl. perfringens* challenge increased the severity of NE (Brennan et al., 2003, 2001b); however, the effect of diet change was tested on the same day (Timbermont et al., 2010), one day after (Gholamiandehkordi et al., 2007), or seven days before challenge (Brennan et al., 2001a, 2003). Chickens fed with a high protein diet for a longer time seems to

reproduce more severe NE, but details of timing have not been determined. Diets containing lower energy to protein ratios can lead chickens to consume more feed and exceed their requirements for protein thereby causing an increase in the nitrogen content of the digesta and excreta (McDevitt et al., 2006). Diets that contains high protein contents or imbalanced profile of amino acids may cause a decreased digestibility in the upper gastrointestinal tract (GIT); thus, these compounds and metabolites become nourishing substrate for the proliferation of clostridia in lower GIT (Lan et al., 2005; Williams et al., 2001). Further, these nitrogenous degradation products raise the pH of the lower GIT which enhance the proliferation of *Clostridium* spp. as well as perturbation of microbiota; meanwhile, it leads to higher water intake of chickens with consequently wet litter containing higher ammonia which promotes the growth of clostridia in litter (Juśkiewicz et al., 2004; Lan et al., 2005; McDevitt et al., 2006).

1.2.3.2 Non-starch polysaccharides

Chickens on wheat, rye, oats, and barley-based diets are more likely to have higher mortality and lower growth rate than chickens on corn-based diets (Branton et al., 1997; Riddell and Kong, 1992) because such grains have high levels of water-soluble non-starch polysaccharides (NSPs) which can increase digesta viscosity and provide substrates for growth of *Cl. perfringens* (Annett et al., 2002; Cooper and Songer, 2010; Dahiya et al., 2006). Traditionally, oligosaccharides were regarded as dietary fiber, but they are grouped as non-starch polysaccharides (Kaldhusdal, 2000). The higher viscosity of digesta leads to a prolonged transit time in the intestine that benefits for perforation of clostridia (Annett et al., 2002). In a NE challenge model, chickens fed with digested maize had mortalities that ranged from 0-12%, whereas chickens fed with barley, rye or

wheat showed mortality of 26-35% (Riddell and Kong, 1992). The broilers on corn-based diet had lower number of *Cl. perfringens* in the intestine than in broilers fed with 50% rye (Craven, 2000). The interaction between NSPs and glycoproteins on the epithelial surface can increase intestinal mucin production (Kleessen et al., 2003) and allow pathogenic microorganisms to adhere to the mucin or tissue and proliferate. NSPs can also increase the water intake of birds resulting in wet litter, which can, in turn, induce sporulation of contaminating *Cl. perfringens* within the litter. Due to their low digestibility, NSPs reach the lower GIT and alter its environment (Reid and Hillman, 1999; Weurding et al., 2001). The adverse effect of cereal combinations (high wheat or barley, low maize) on NE was exacerbated when animal proteins were included in the diet (Kaldhusdal and Skjerve, 1996). *Cl. perfringens* lacks the ability to produce 13 out of the 20 essential amino acids; therefore, its growth is facilitated in an environment with high content of protein, particularly fish meal.

1.2.3.3 Other changes

The size of the feed particles has been shown to affect the number of *Cl. perfringens* in the intestine (Engberg et al., 2002). In Engberg's study, it was found that the counts of *Cl. perfringens* were significantly higher in the ceca and rectum of chickens on mash-fed diet because there was more undigested substrate left for its growth. Chickens on pellet-fed diet had a lower mortality and improved feed conversion ratio than chickens fed with the mash-fed diets because the disintegration of pellets in upper GIT, especially gizzard. Therefore, the dominating bacteria which are lactic acid-producing bacteria, in particular lactobacilli (Barnes et al., 1972; Engberg et al., 2000), can utilize them instantly and offer certain protection from diarrhea-causing pathogens

belonging to the gram-negative flora. Manipulation of feeding schedules, including sudden major changes to feed composition and withdrawal of feed for 8–12 hours prior to challenge, seemed to be a key part of some experimental disease induction models (Feng et al., 2010; Keyburn et al., 2006) and was likely to significantly disrupt the GIT microbiota. One of the changes identified following mycotoxin treatment was a reduction in the segmented filamentous bacteria and this may have significance for immune development (Snel et al., 1995; Talham et al., 1999). Some dietary factors that affect the incidence of NE were listed in McDevitt's study (Table 1.5).

Numerous antimicrobials carry out effects of reducing intestinal level of in *Cl. perfringens* broilers and improving the growth rate; however, a role of growth suppression played by *Cl. perfringens* under the antimicrobial administration had been emphasized by several studies (Hofshagen and Kaldhusdal, 1992; Stutz and Lawton, 1984). Bacterial enteritis has been recognized as a process by which the intestinal bacterial population changes in response to the environmental changes in the intestine (Collier et al., 2003; Fukata et al., 1991; Netherwood et al., 1999). Studies regarding the intestinal microflora in normal and clinically affected broilers suggested that the colonization of *Cl. perfringens* in the ceca played a key role in the onset of NE (Cooper et al., 2013; Long et al., 1974). Because the presence of antibiotic-resistant strains and their transmission to human had potential to threaten food safety and public health, the prophylactic use of AGPs which was considered as one of the sources of antibiotic-resistant strains was banned in some countries. Consequently, it appeared to result in an increasing incidence of clinical or subclinical NE (Fasina et al., 2016; Wilson et al., 2005).

1.3 THE SYSTEM DYNAMICS MODEL

1.3.1 Modeling and System thinking

Models are instruments applied to investigate the interactions of indicated elements in the complex dynamic systems observed in nature. To build up a model presenting a dynamic system, knowledge of experts and statistical methods are incorporated to recreate observed behaviors and find patterns in the measured data. By simulating the model, its states under various conditions can be described or/and predicted (Simidjievski et al., 2016). Models are used to interpret the results of study, whether it is a physical model used for experimentation, a statistical model used to estimate the relationships between variables, or a conceptual model about how elements are connected. It is a simple way to represent and understand an object, phenomenon, or system. The core of systems thinking is aimed to improve the quality of perceiving a system, its parts, and the interactions within and between levels. A more explicit model with repeatability and obvious assumptions can be established with the system thinking (Peters, 2014). In Peter's paper, there are several theories that provide the application of the system thinking models and frameworks with basic concepts of several disciplines, such as general systems theory, a way of finding a general theory to explain systems in all fields of science, or cybernetics, a field of study of the communication and control of regulatory feedback in both living and non-living systems. There are also several methods of modeling, including social network analysis, which uses graphical methods to demonstrate relations between objects, and system dynamics modeling, which is not a single method, but an approach that uses a set of tools to understand the behavior of complex systems over time. With a variety of useful tools, such as causal loop diagrams,

stock and flow diagrams, participatory impact pathways analysis, and process mapping, models of system thinking are mapped out in a qualitative, quantitative or both descriptive ways.

1.3.2 System Dynamics model

The System Dynamics (SD) model launched in 1960s by Jay Forrester incorporated systems thinking and computer engineering in his book, *Industrial Dynamics* (Forrester, 1961). System dynamics accepts the complexity, nonlinearity and feedback loop structures that are inherent in a system, and represents the real world (Forrester, 1994). It was demonstrated that a central tenet of system dynamics was the complex behaviors of organizational and social systems came from the result of ongoing accumulations, such as people, material, information or biological states, and balancing and reinforcing feedback mechanisms. System dynamics offered the practical application of the concepts of accumulations and feedbacks in the form of computerized models; meanwhile, by testing the alternative polices and scenarios, the questions of what if and why could be answered (Homer and Hirsch, 2006). An interlocking set of differential and algebraic equations developed from a broad spectrum of relevant measured and experiential data is contained in a system dynamics model, and as it becomes completed, hundreds of such equations with the appropriate numerical inputs are included. The process of modeling is an iterative procedure of scope selection, hypothesis generation, causal diagramming, quantification, reliability testing, and policy analysis (Sterman, 2000). As in the paper of Homer and Hirsch (Homer and Hirsch, 2006), it was described that the refinement process continues until the model satisfies requirements concerning

its realism, robustness, flexibility, clarity, and ability to reproduce historical patterns as well as generate useful insights.

In Forrest's paper of 1994 (Forrester, 1994), a process of system dynamics was illustrated (Figure 1.1). First of all, the relevant system is described with a hypothesis for how the system may behave. This description is then translated into the level and rate equations of a system dynamics model. Followed by simulating the model with the system dynamics software, the equations pass the logical criteria of an operable model, such as well-defined variables and consistent units of measurement. Consequently, simulations may help to clarify the description of system in the first step as well as the refinement of the equations in the second step and show how the problem under consideration is generated in the real system. At each step, consideration and refinement occur retrospectively to prior steps. After simulations of the model, it is important to test alternative policies (or strategies) and structures which may come from intuitive insights generated during the previous stages, from experience of the analysts, from proposals provided by people in the operating system, or by an automatic testing of parameter changes. The last two steps require a consensus from the team to implement modified policies or new policies which may counter traditional practices, and also intense education with sufficient time to overcome resistance.

1.3.3 The application of SD models in population health

The studies of human population health applied with the SD model have been conducted since the 1970s (Homer and Hirsch, 2006). Topic areas fell in the following categories: [1] Epidemiology of chronic or contagious diseases, such as heart diseases (Luginbuhl et al., 1981), human immunodeficiency virus (HIV) (Roberts and

Dangerfield, 1990), dengue fever (Ritchie-Dunham and Galvan, 1999), drug-resistant pneumococcal infections etc. (Homer et al., 2000); [2] Substance abuse epidemiology covering heroin addiction (Nagel, 1978), cocaine prevalence (Homer, 1993), and tobacco reduction policy (Levy et al., 2006); [3] Patient flows in emergency and extended care (Royston et al., 1999; Wolstenholme, 1999); [4] Health care capacity and delivery in areas as planned by certain organizations (Hirsch and Miller, 1974) and as affected by natural disasters or terrorist acts(Caulfield, 1977); [5] Interactions between health care or public health capacity and disease epidemiology (Hirsch G. et al., 2004; Hirsch and Immediato, 1999).

The SD model has recently been applied to various research studies regarding animal diseases and their occurrence related to meat production, food supply chains or ecological sustainability. A study of analyzing the supply chain behaviors used the SD model to simulate the scenarios of the shortages in upstream supply capacity and unpredictable consumer behavior under bird flu crisis during the period from 2005 to 2006 in France (Le Hoa Vo, and Thiel, 2007). As a visualizing tool to present the movement of the entire production chain, the system dynamics model enabled the integration of important factors at each breeding level that will affect the number of fattening pigs as well as the interactions in the supply chains in Thailand (Piewthongngam et al., 2014) and Vietnam (Nguyen et al., 2014). By integrating the SD model of Susceptible-Infected-Removed (SIR) model and economic model, the feedbacks that exist between the evolution of disease and producer responses to an outbreak of animal diseases were captured. Two direct impacts, which are significant mortality and reducing demands, and their interactions, such as marketing decisions or holding of

animals, were also identified in this type of SD model (Rich, 2007). Utilizing the SD model to build up a decision support system in tropical and subtropical regions estimating the increasing production of small ruminates and pressure on livestock system could benefit to address issues such as greenhouse-gas emissions and the effects of climate change on livestock system (Tedeschi et al., 2011). A spatial group model building incorporated with the SD model was established to investigate the influence of dynamic socio-economic, cultural, and ecological factors of the occurrence of East Coast Fever in beef cattle in Zambia (Mumba et al., 2017).

1.3.4 Susceptible-Infectious-Recovered/Removed (SIR) model

The SIR model, a type of compartmental model, divides individuals into categories, susceptible, infectious (infected) and recovered/removed, based on their disease status (Figure 1.2). It is commonly used as a first attempt to characterize outbreaks or infections quickly and require less computational resources (Daughton et al., 2017). The susceptible (S) population is individuals that are at risk of infection. The infectious (I) population are individuals experiencing the illness and having clinical signs. The recovered/removed (R) populations are individuals that have completed the infection and ended with immunity or death (Figure 1.3). As the infectious disease progresses, the number of individuals in each compartment changes with time; however, the overall number in the three compartments remains constant (S+I+R=1) with an initial conditions that number of R starts with zero while number of S and I are accumulating (S(0)>0, I(0)>0, R(0)=0) (Keeling and Rohani, 2008). There are two factors determining whether an epidemic occurs or the infection fails to invade. One is the threshold phenomenon which means the initial proportion of susceptible in the population must exceed a certain

number which depends upon the infectivity, recovery rate and death rates peculiar to the epidemic. Another is the virulence of the causative organism which has to gradually decrease during the epidemic (Kermack and McKendrick, 1927). The basic reproductive ratio (R_0), defined as the average number of secondary cases arising from an average primary case in an entirely susceptible population, is also one of the most important qualities in epidemiology. It can be used to re-express the threshold phenomenon because normally any infection requires successful transmission to more than one new host to spread out (Keeling and Rohani, 2008). R_0 must be maintained below 1 to stop the epidemic (Lipsitch et al., 2003). Generally, the disease that has a higher transmission rate (β) and a longer infectious period ($1/\gamma$) has a higher reproductive ratio ($R_0 = \beta/\gamma$) (Keeling and Rohani, 2008).

For studying the spread of infectious diseases, the epidemic dynamics is applied as an important method because it is based on the specific property of population growth, spread rule of infection, and the related factors of social interactions. The mathematical models constructed to reflect the dynamics of disease can be simulated to show the behaviors for further analyses. These results can help to predict the epidemics, determine key factors of disease spreading, and seek optimal strategies of control as well as prevention (Keeling and Rohani, 2008; Lipsitch et al., 2003). It was recommended that, without latent periods, if the recovered individuals gain immunity to the causative agent, the SIR model would be applicable, such as influenza and measles. If the infected ones cannot obtain immunity to the disease, the susceptible-infectious-susceptible (SIS) model would be more applicable because the infected individuals will be recovered but not gain the immunity, such as tuberculosis and syphilis. With latent periods, the individuals are

categorized in the exposed (E) compartment; thus, the susceptible-exposed-infectious (SEI), susceptible-exposed-infectious-recovered (SEIR) or susceptible-exposed-infectious- susceptible (SEIS) model are applicable, such as streptococcal infections (Badshah et al., 2013; Keeling and Rohani, 2008).

1.3.5 Development of the SD model

In comparison with other types of models established by mathematical and statistical methodology, the SD model shows the equations instantly and its development transparently. The value of SD modeling is best explained by way of illustration (Homer and Hirsch, 2006). The principle of SD model is based on system behaviors determined by causal structure because variables inside are linked. These combinations of links carry out feedback loops which are either termed reinforcing (positive) or balancing (negative) loops depending on the aggregate polarity of each link (Figure 1.4). Positive loops tend to amplify any disturbance and to produce exponential growth, whereas negative loops tend to counteract any disturbance and to move the system towards an equilibrium point or goal. These causal loop diagrams (CLDs) support the illustration of system dynamics; moreover, the more explicit of endogenous variables and circular causality becomes, the clarity of problem is understood (Tedeschi et al., 2011). The stock and flow diagrams are distinguished from causal loops in the SD model. Stocks are accumulations over time which flows increase or decrease them (Figure 1.5). A stock is like a bathtub filled with water so that the difference between the inflow of water tap and the outflow of drain determine the level of water inside (Sweeney and Sterman, 2000). It is the classic example for introducing the concept of the stocks and flows. Once the relative level of water reaches the capacity of the bathtub, a feedback mechanism is created by changing

the flow rate; meanwhile, if the information is delayed that the bathtub may overflow before the action is taken (Tedeschi et al., 2011). The basis of SD models are formed by the combination of causal loops, stocks, flows, and delays which depict fundamental distinct patterns of behavior, including exponential growth, goal seeking, and oscillation, and combination of these fundamental behaviors, such as S-shaped growth (Figure 1.6) (Tedeschi et al., 2011).

According to Tedeschi (2011), there are a series of grouped first-order differential equations, a set of parameters and a vector of non-linear functions represented in SD models. The examination of effects coming from parameter and structural changes of the system can be supported by the numerical simulation of the system which forces algebraic rigor upon the model. A *dynamic hypothesis* is what SD models typically focus on and is the origin of a problematic behavior. It is modelling the elements that are necessary to explain a particular phenomenon rather than all relevant elements of a system. The general dynamic tendencies, which considers the system as a whole is unstable or stable, growing, self-correcting, or in equilibrium under certain condition, is valued. Therefore, SD models are advantageous with better understanding of the pattern of adjustment over time in response to various interventions or policy. In addition, an important feature of the process of SD modelling is that it works at very abstract levels with focus on problem structures and permits analysis in situations where data is uncertain or simply unavailable.

1.3.6 Validation of the SD model

1.3.6.1 Test procedures

Tests for model structure. Because the structure is the foundation for model behavior, the first test in validating a SD model is whether the structure of the model matches the structure of the system being modeled (Shreckengost, 1985). There are elements that are not quantified or are unavailable, but as they contribute significantly to confidence to the system being modeled, they must be developed from reasonable parameter value and be consistent with supporting data (Sterman, John D, 1984). In order to match the purpose for designing the model, model boundaries have to include all the important factors affecting the behavior of interest as possible. However, as the purpose can shift during the process, changes of the boundaries can be made. Besides, boundary charts listing the endogenous, exogenous or excluding variables can help to decide the model boundary (Sterman, 2002). The utility of the SD model as a policy evaluation tool and user confidence is built up on the ability of a model to function properly under extreme conditions. The point of applying extreme conditions is that model validity is enhanced when the originally designed region is extended and thus the model generates plausible behaviors outside the initial region (Shreckengost, 1985).

Test for model behavior. The higher the similarity between the model and the system of interest exists, the higher degree of confidence the model reaches. If the initial conditions of the model match the past state of the system being modeled at some time, the behavior of model should parallel the historical data simultaneously (Sterman, 1984). When an assumption is deleted in the model, its behavior cannot replicate the behavior of the real system, improperly specified or incorrectly assigned values can be omitted. This

kind of anomalous behavior test sometimes convincingly contributes to model validity. Under the criterion of sensitivity test, small and reasonable changes in parameter values should not produce radical behavior changes because any sensitivity exhibited by the model has to be consistent with observed behavior in the real system (Shreckengost, 1985). The SD models should be able to reproduce the behavior of other examples of systems in the same class as the model; thus, confidence is enhanced not only because the complementary systems can contribute to the robustness of the model developed for a particular member of the family, but also because the differences among the members can be explicitly identified and defined (Sterman, 1984).

Tests for policy implication. System improvement, changed behavior prediction, boundary adequacy, and policy sensitivity tests are included, and their functions are testing whether a real system's response to a policy change would replicate the response to the policy change predicted by a model. These tests tend to be long term and reflect a different perspective in the application in comparison with previous ones (Shreckengost, 1985). Extreme policy test introduces radical policies into the model to see if the behavior of the model is consistent with what would be expected under these conditions.

1.3.6.2 Sensitivity analysis

The sensitivity analysis is helpful to build confidence in the model by studying the uncertainties associated with parameters, especially those representing qualities. Many of these parameters used in the model are hard to measure to a great deal of accuracy or often change in the real world. Therefore, while building a SD model, the modeler is sometimes uncertain about the parameter values chosen and must use estimates (Breierova and Choudhari, 1996). By using the sensitivity analysis, the modeler

can determine what level of accuracy is necessary for a parameter to make the model sufficiently useful and valid. In SD modeling, the *behavior mode sensitivity*, which represents changes in output behaviors (e.g. S-shaped growth), is more important that the results of changes in parameter values (Sterman, 2000). When the model shows insensitivity after testing, using an estimate for the parameter may be possible than using a value with greater precision. The sensitivity analysis can indicate which parameter values are reasonable to use in the model. If the model's behaviors are similar with the expectation from real world observations, it indicates to a certain level that the parameter values reflect the real world (Breierova and Choudhari, 1996).

By experimentally inputting a wide range of values, insights into behaviors of a system in extreme situations can be seen. If the system behavior greatly changes for a change in a parameter value, the parameter is revealed as a leverage point that the behavior mode of the system can be significantly influenced by the parameter with specific value. The graphs representing the behavior of the system may be changed by a specific parameter; however, significant changes in behavior do not occur for all parameters. SD models are generally insensitive to many parameter changes because the structure of the system has more influence on the behavior of the system than parameter values do (Breierova and Choudhari, 1996).

Table 1.1 Characteristics of important *Eimeria* spp. infecting chickens

Eimeria	**Location of developmenta**[abc]	**Pathogenicity**[bd]	**Immunogenicity**[d]	**Sporulation time* (HR)**[a]
E. acervulina	Duodenum, Jejunum, Ileum	Low to moderate	Moderate	17
E. brunetti	Lower small intestine, basis of ceca, and rectum	Moderate to high	High	18
E. maxima	Jejunum, Ileum	Moderate to high	High to very high	30
E. mitis	Ileum	Low	Moderate	15
E. necatrix	Jejunum, Ileum, Caeca	High to very high	Low	18
E. praecox	Duodenum, Jejunum	Low	Moderate	23
E. tenella	Caeca	High to very high	Low	18

(Adopted from [a]McDougald and Long, 2003, [b]Fornace et al., 2013; [c]Shivaramaiah et al., [d]2014; Witcombe and Smith, 2014)); *Minimum

Table 1.2 Individual pathogenicity and gross lesions of *Eimeria* spp.

Eimeria	**Pathogenicity**	**Gross lesions**
E. acervulina	▪ The outline of lesion is seen on the mucosa surface on the 4^{th} - 9^{th} day postinfection. ▪ Heavier infections of some strains have produced 70% mortality in some breeds of chickens. ▪ Recovery of the normal rate of lay requires about 3 wk. ▪ Larger inocula produced increasingly severe effects. Single and multiple doses of 5 million or more oocysts caused 6 to 75% mortality.*	▪ It is usually in the epithelium with no extension into the lamina propria. ▪ Maximal damage may be seen 5 days postinfection. ▪ Both schizonts and gametocytes locate above the nucleus of the epithelial cell.
E. brunetti	▪ 10-30% of mortality has sometimes been reported. ▪ It is currently being recovered infrequently from broilers in the US.	▪ Numerous young schizonts cand be found at 3.5days. ▪ By the end of day 4, large numbers of released merozoites begin to parasitize the epithelium, and sexual stages establish themselves with inpoint lesions in the lower small intestine and ceca. ▪ Both schizonts and gametocytes locate above the nucleus of the epithelial cell.
E. maxima	▪ Slight to moderate mortality has been reported from both field and experimental infections, although some strains produce none. ▪ Subacute infections may induce depigmentation and poor carotenoid absorption in birds. ▪ Brackett and Bliznick (1950) observed a mortality of 35% in one infected with 500,000 ocysts each, but there were no deaths in another group.*	▪ Minimum tissue damage occurs with the first two asexual cycles. ▪ Not until sexual stages develop in the deeper tissues on days 5-8 of the cycle does tissue damage become severe. ▪ The gametocyte develops beneath the host cell nucleus, and developing schizonts usually parasitize the host cell above the nucleus.

Table 1.2 (continued)

Eimeria	Pathogenicity	Gross lesions
E. mitis	▪ Although it has often been regarded as nonpathogenic, some morbidity in young chicks has been reported. ▪ Subclinical and pathogenicity would be classed as mild.	▪ By the end of day 4, schizonts containing 24- 60 merozoites appear scattered among epithelial cells of the villi. ▪ Schizonts, microgametes, and macrogametes may develop superficiall or beneath the nuclei of epithelial cells.
E. necatrix	▪ Mortality begins on day 5, shows greatest severity on day 7, and extends to day 12. ▪ *E. necatrix* infection produces greater mortality than any species with the exception of *E. tenella*. ▪ Mortality usually occurs early in the life cycle, but signs of morbidity may continue for at least 1 wk longer than with other species. ▪ Weight loss becomes apparent on day 6 and reaches a maximum on days 7 to 9. ▪ Natural attacks in field flocks typically occur 1- 3 wk later than with *E. tenella*. ▪ Brackett and Bliznick (1950, 1952) found that in 3-week-old chicks, 25, 000 oocysts caused a mortality of 87%, while in 4-week-old chicks, 18,000, 37,000, 75,000 and 150,000 oocysts caused mortalities of 8, 75, 85 and 61%, respectively.*	▪ The first gross lesions appear 1.5-3 days postinfection, when 1st generation merozoites are released. ▪ The prominent colonies of 2nd generation schizonts are best seen on the 6th day. ▪ Invasion of the cecal epithelium by 2nd generation merozoites beginning on day 5 produces little damage. ▪ Parasites produced in the asexual generations are superficial to the nucleus of the epithelial cell; developing gametocytes may occur either above or below the nucleus.

Table 1.2 (continued)

Eimeria	Pathogenicity	Gross lesions
E. praecox	▪ This species produces sufficient morbidity to warrant its inclusion in planned immunization programs.	▪ Small pinpoint hemorrhages are seen from the mucosal surface on the 4th and 5th days of infection, but no lesions are visible on the serosal surface. ▪ Parasites establish themselves either above or below the nucleus of the epithelial cell.
E. tenella	▪ Flock morta lity of 20% or more has occurred within a period of 2-3 days. Blood loss from cecal lesions, diarrhea, huddling, and a characteristic odor may be noted shortly before mortality begins. ▪ Depressed growth rate or actual weight loss may occur, with maximum effects occurring on the 7th day postinfection. ▪ Coccidiosis is a self-limiting disease; most birds that survive for 8 days will recover. ▪ Oocysts begin to appear the 7th day postinfection, reach a peak of several million per bird on the 8th day, and are reduced number on the 9th and following days. ▪ Waletzky and Hughes (1949) found that 20,000 oocysts produced 18%; mortality and 100,000 oocysts 36% mortality in 4-weck-old chicks.*	▪ Presence of characteristic bleeding from cecal walls on days 5 and 6 of the cycle or presence of hardened cheesy cores in later stages suggests *E. tenella* infection. ▪ The first gross changes with some enlargement of the ceca and appearance of small a reas of hemorrhage are noted on the 3rd day. ▪ Regeneration of the epithelium and glands is complete by day 10 in light in fections; healing in severe infections may take 3 wks. ▪ Both asexual and sexua l forms of parasites develop beneath the nuclei of epithelial cells.

(Adopted from *Levine, 1961; McDougald and Long, 2003)

Table 1.3 The prevalence of prevalent *Eimeria* spp. in countries with available information

Country	Prevalence in prevalent *Eimeria* species in commercial flocks (%)						
	E. tenella	***E. acervulina***	***E. necatrix***	***E. brunetti***	***E. praecox***	***E. mitis***	***E. maxima***
Australia[1] (fecal sampling)	9	67	*	*	*	58	46
Belgium[8]	80	100	--	--	90	40	50
Southern Brazil[2] (fecal sampling)	54.6	63.3	24.3	13.1	25.1	38.6	63.7
France[9]	35	67.5	--	--	5	5	22.5
India[3] (fecal sampling)	67.3(N) 57.5(S)	45.8(N) 12.7(S)	43(N) 14.9(S)	3.7(N) 0.7(S)	32.7(N) 0(S)	58.9(N) 29.9(S)	28(N) 9(S)
Italy[9]	80	93.3	--	--	60	20	33.3
Norway[3] (fecal sampling)	77.05	100	1.64	--	9.84	--	24.59
Northern Jordan[4] (post-mortem sampling)	39	3	12	12	--	1	10
Pakistan[5] (post-mortem sampling)	21.4	4.21	11.92	--	--	2.1	5.61
Romania[6] (fecal sampling)	61	91	--	--	13	--	22
Spain[8]	70	100			50	10	60
South[7a] Korea (fecal sampling)	62.5	87.5	31.3	59.3	37.5	31.3	31.3

Table 1.3 (continued)

Country	Prevalence in prevalent *Eimeria* species in commercial flocks (%)						
	E. tenella	***E. acervulina***	***E. necatrix***	***E. brunetti***	***E. praecox***	***E. mitis***	***E. maxima***
The U.S.[10] (litter sampling)	28.4	90.6	0.4	2.3	--	--	86.2

* No precise number

-- No data

(N) Northern

(S) Southern

[a] Percentage of *Eimeria* spp. in positive flocks (n=61)

[1]Godwin and Morgan, 2015(broilers), [2]Moraes et al., 2015 (broilers), [3]Chengat Prakashbabu et al., 2017 (breeders, broilers, layers), [4]Haug et al., 2008 (broilers), [5]Al-Natour et al., 2002 (broilers), [6]Sharma et al., 2015 (backyard and commercial flocks), [7]Györke et al., 2013 (broilers), [8]Lee et al., 2010 (broilers, layers), [9]Pagès et al. 2015 *Eimeria*-prevalence-study-in-Europe/South Africa (broilers), [10]Jeffers, 1974a(broilers)

Table 1.4 Key contributing factors for the occurrence of wet litter

Key contributing factor	References
Condensation on walls, ceilings and in-shed equipment	Hermans et al., 2006
Drinker design	Bilgili et al., 1999; Shepherd and Fairchild, 2010; Tucker and Walker, 1992
Diarrhea	Collins et al., 1989; Neill et al., 1984
Farm biosecurity and cleaning practices	Hermans et al., 2006
Litter/bedding material type	Bilgili et al., 2009; Bruce et al., 1990; Davis et al., 2010; Meluzzi et al., 2008; Shepherd and Fairchild, 2010; Tucker and Walker, 1992
Lighting equipment or program	Meluzzi et al., 2008
Litter moisture content/ water holding capacity	Bilgili et al., 2009; Shepherd and Fairchild, 2010
Excess litter depth	Ekstrand et al., 1997
Insufficient litter depth	Hermans et al., 2006; Tucker and Walker, 1992; Weaver and Meijerhof, 1991
Normal water excretion	McIlroy et al., 1987; Tucker and Walker, 1992; van der Hoeven-Hangoor et al., 2013a, 2013b, 2013c; Weaver and Meijerhof, 1991
Increased water excretion	Bruce et al., 1990; Collett, 2012; Eichner et al., 2007; Francesch and Brufau, 2004; LaVorgna et al., 2014; McIlroy et al., 1987; Shepherd and Fairchild, 2010; Tucker and Walker, 1992; van der Hoeven-Hangoor et al., 2013a, 2013b, 2013c
Season	Bruce et al., 1990; McIlroy et al., 1987 Hermans et al., 2006; McIlroy et al., 1987; (Wang et al., 1998)
Stocking density	McIlroy et al., 1987; Tucker and Walker, 1992
Temperature and relative humidity of the house	Payne, 1967; Tucker and Walker, 1992 Bruce et al., 1990; Hermans et al., 2006; McIlroy et al., 1987; Shepherd and Fairchild, 2010; Tucker and Walker, 1992; (Wang et al., 1998)
Insufficient shed ventilation/ Air exchange	Hermans et al., 2006; Tucker and Walker, 1992; Weaver and Meijerhof, 1991

(Adopted from Dunlop et al., 2016b)

Table 1.5 Dietary factors affect the incidence of NE

Factor/ compound	**Mechanism References**	**References**
Nitrogen content	High concentration as a substrate clostridium and transport to lower GIT	Kocher et al., 2003
Nitrogen digestibility	Poor digestibility allows transport to lower GIT, substrate for clostridia	Lan et al., 2005; Williams et al., 2001
Amino acid digestibility	Poor digestibility allows transport to the lower GIT, substrate for clostridia	Williams et al., 2001
Non-starch polysaccharides (NSP)	Transport to lower GIT, changes in viscosity, alteration of hydration in GIT, interaction with villi, substrate for microflora	Iji and Tivey, 1998; Juśkiewicz et al., 2004
Types of starches	Resistant starches transport to lower GIT, substrate for microflora	Svihus et al., 2005
Antioxidants	Up-regulate the genes associated with immune response, reduce oxidative damage of the GIT by free radical species	Flachowsky and Peter F. Surai, 2003
Antinutrients	Interact with GIT epithelial tissue, damage tissue	Astley and Finglas, 2016
Vitamins and minerals	Alter the gene expression for immune response and tissue synthesis and secretion of hormones and enzymes	Flachowsky and Peter F. Surai, 2003
Temperature and time of processing	Alter availability of nutrients, produce toxic compounds, reduce antinutrient compounds	Svihus et al., 2005; Clarke and Wiseman, 2005
Toxins	Alter nutrient demands and energy requirement, may promote free radicals within the GIT	Flachowsky and Peter F. Surai, 2003
Particle size	Alter GIT, may interact with microflora, transport of nutrients to the lower GIT	Kaldhusdal and Skjerve, 1996
Enzyme	Alter availability and balance of nutrients supplements and non-nutrients and anti-nutrients, produce compounds that may influence microbial attachment and growth	Acamovic, 2001; Bedford, 2000
Mycotoxins	Alter the GIT, produce toxins and alter nutrient requirements	Fink-Grernmels, 1999
Dietary organic and inorganic acids	Alter pH in the GIT and thus microflora	Dibner and Buttin, 2002
Diet and nutrient intake	Alters gene regulation and transit/residence time in the GIT	Kita et al., 2005

(Adopted from McDevitt et al., 2006)

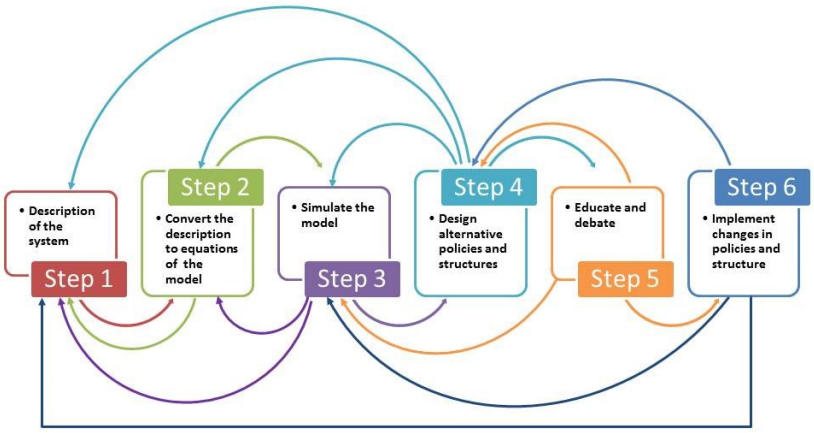

Figure 1.1 The system dynamics steps from problem symptoms to improvement

First of all, the relevant system is described with a hypothesis for how the system may behave. This description is translated into the level and rate equations of a system dynamics model. Followed by simulating the model with the system dynamics software, the equations pass the logical criteria of an operable model, such as well-defined variables and consistent units of measurement. Consequently, simulations may help to clarify the description of system in the first step as well as the refinement of the equations in the second step and show how the problem under consideration is generated in the real system. At each step, consideration and refinement occur retrospectively to prior steps. After simulations of the model, it is important to test alternative policies (or strategies) and structures which may come from intuitive insights generated during the previous stages, from experience of the analysts, from proposals provided by people in the operating system, or by an automatic testing of parameter changes. The last two steps require a consensus from the team to implement modified policies or new policies which may counter traditional practices, and also intense education with sufficient time to overcome resistance. (Adopted from Forrester, 1994)

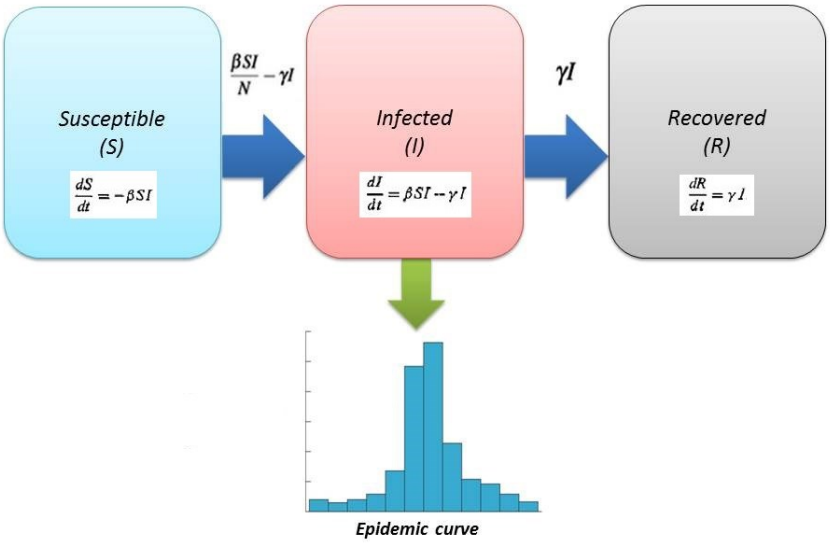

Figure 1.2 The compartments and equations of SIR model

Individuals move between three compartments, S (susceptible), I (infectious/ infected), and R (Recovered), by transmission rate (β), removal or recovery rate (γ) and infectious period (1/γ). For an infectious disease with an average infectious period (1/γ), its basic reproductive ratio (R_0), defined as the average number of secondary cases arising from an average primary case in an entirely susceptible population, is determined by β/ γ. The known epidemic curve is resulted by the number of infected individuals at any given time. (Adopted from Daughton et al., 2017; Keeling and Rohani, 2008)

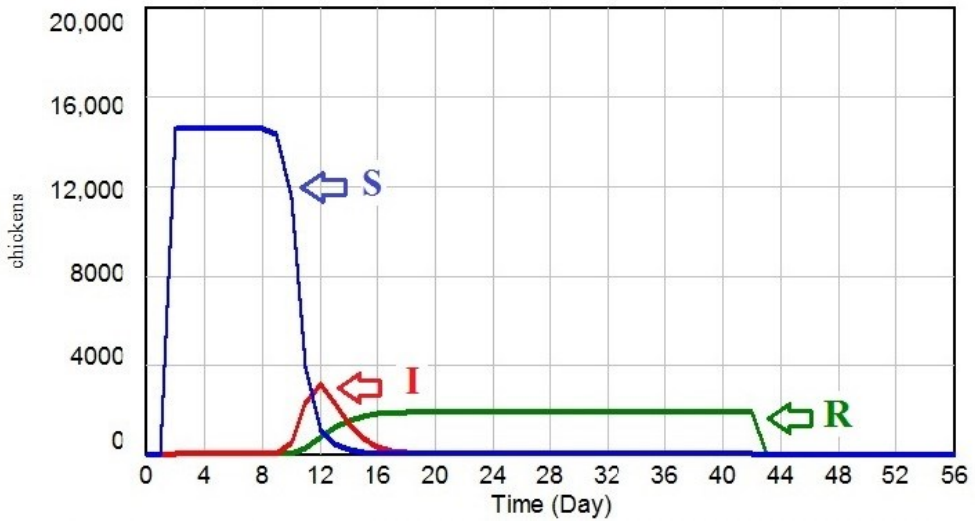

Figure 1.3 The graph of SIR model

It is a time-evolution of model variables with an initially entirely susceptible population and a single infectious individual. The susceptible (S) population is individuals that are at risk of infection. The infected (I) population is individuals experiencing the illness and having clinical signs. The recovered/removed (R) populations are individuals that have completed the infection and ended with immunity or death.

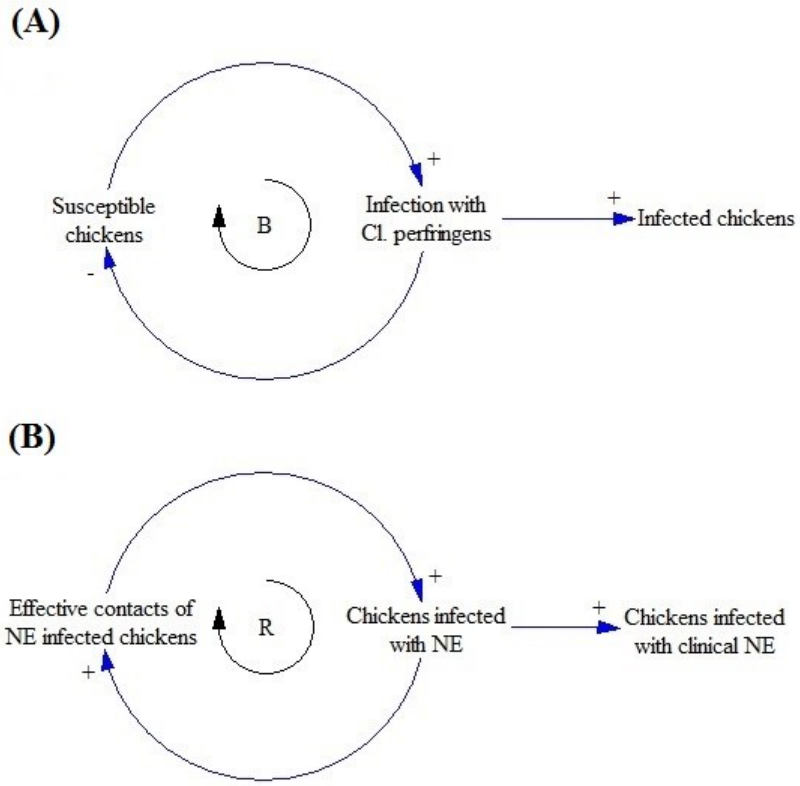

Figure 1.4 A causal loop diagram

The principle of SD model is based on system behaviors determined by causal structure because variables inside are linked. These combinations of links carry out feedback loops which are either termed reinforcing (R) (positive) or balancing (B) (negative) loops depending on the aggregate polarity of each link. Positive loops tend to amplify any disturbance and to produce the exponential growth, whereas negative loops tend to counteract any disturbance and to move the system towards an equilibrium point or goal. This figure demonstrates a balancing loop of susceptible chickens which continuously become infected with *Cl. perfringens* and then enter into the stage of infected chickens(A); whereas, a reinforcing loop of effective contacts of NE infected chickens are becoming infected and showing clinical signs (B).

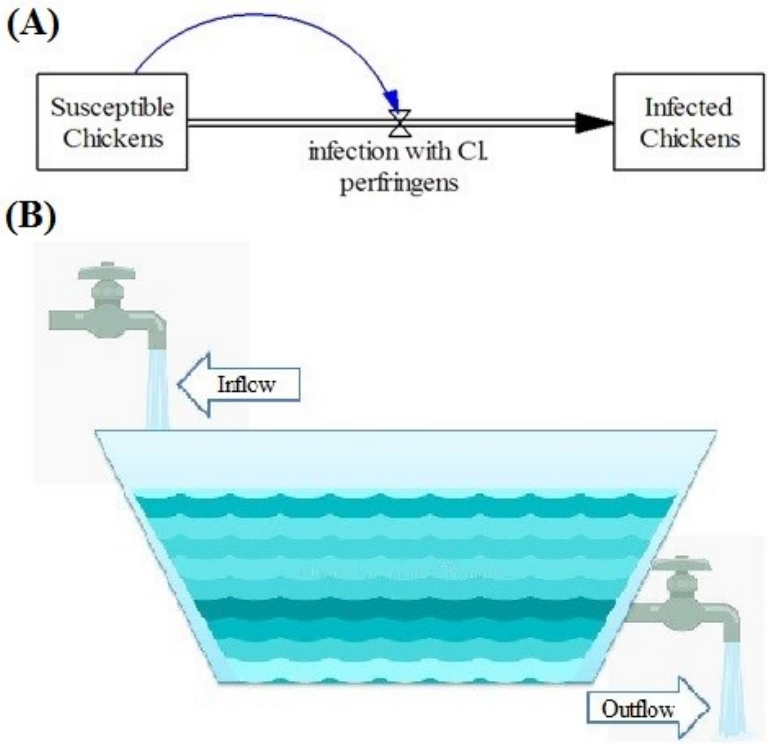

Figure 1.5 A stock and flow diagram

The stock and flow diagrams are distinguished from causal loops in the SD model. Stocks are accumulations over time which flows increase or decrease them. This figure shows the accumulation of chickens infected with *Cl. perfringens* from susceptible chickens is adjusted by the rate of chickens get infected over time (A). A stock is like a bathtub filled with water so that the difference between the inflow of water tap and the outflow of drain determine the level of water inside (B) (Adopted from Sweeney and Sterman, 2000). It is the classic example for introducing the concept of the stocks and flows. Once the relative level of water reaches the capacity of the bathtub, a feedback mechanism is created by changing the flow rate; meanwhile, if the information is delayed that the bathtub may overflow before the action is taken (Tedeschi et al., 2011).

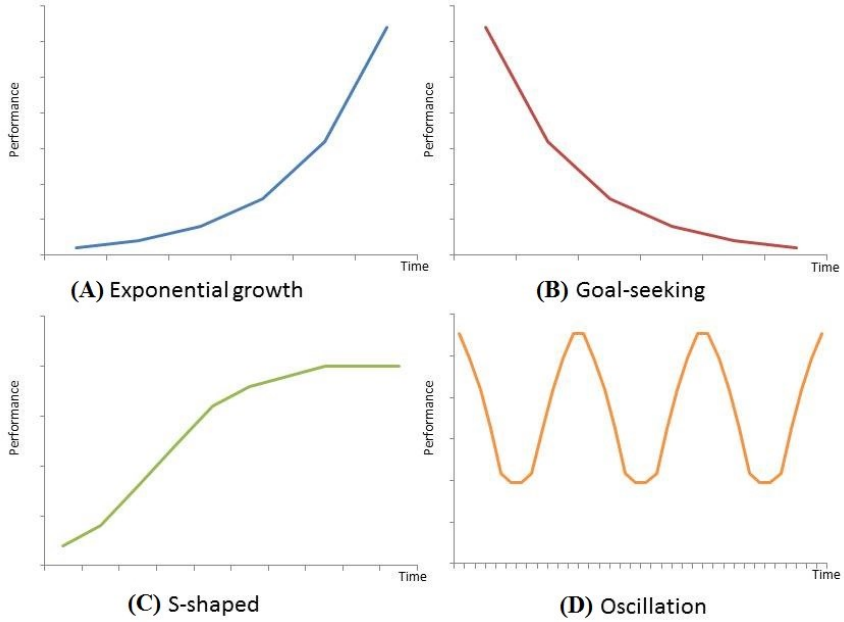

Figure 1.6 Characteristic patterns of system

The basis of SD models are formed by the combination of causal loops, stocks, flows, and delays which depict fundamental distinct patterns of behaviors: Exponential growth is a common pattern of behavior generated by the reinforcing (positive) loops(A). Goal seeking behavior comes from the balancing (negative) loops (B). S-shaped pattern begins with an initial exponential growth followed by a take-over of the goal-seeking behavior (C). Oscillation usually needs at least 2 stocks in a process with the impact of delays which form the degree of oscillation (D). (Adopted from Kirkwood C.W., 1998)

CHAPTER II

MATERIAL AND METHOD

2.1 MODEL DEVELOPMENT AND METHODOLOGY

2.1.1 Data sources

Data collected for the development of the SD model was drawn from several sources: 1) literature regarding NE and avian coccidiosis, including experimental models and field studies, 2) publications or guidelines collected from poultry industry, 3) reports of the USDA annual census and monitoring plans, and 4) opinions of veterinarians and experts in the broiler industry. We calibrated the model by using an iterative process of testing parameter values and observed data of available experimental models with the simulated behaviors of the model.

2.1.2 The causal loop diagrams (CLDs)

Based on the knowledge regarding the SD modeling and NE, our SD model was constructed with a SIR model which represented the disease epidemiology over time and the relationships with its predisposing factors of concerns, particularly the avian coccidiosis. Without application of medication, there will be a removed population either dying from NE or harvested for processing. The SIR model was also applied to avian coccidiosis and coinfection of clinical NE and coccidiosis.

At the first stage of model development, the CLDs were built and categorized the major groups of feedback loops which showed the correlations and variations of

susceptible, exposed, infected and removed chickens. The reinforcing loops (R1 to R4) (Figure 2.1), known as the contagion loops in the SIR model, showed chickens that were becoming infected by pathogenic *Cl. perfringens* and *Eimeria* spp. under the exposure to effective contacts (R1 and R3). The chickens infected with pathogens increased and consequently separated into two populations, subclinical and clinical infected chickens. During the progress of disease, clinical infected chickens increasingly turned into the subclinical infection (R2 and R4) or directly harvested at the end of grow-out period. In the balancing loops (B1 to B11), known as the depletion loops in the SIR model, as the chickens gradually were infected with pathogens, the populations of susceptible and effective contacts declined (B1 and B5). Meanwhile, whenever populations of subclinical infection (B2 and B7), clinical infection (B3 and B6) and coinfection (B9 and B10) increased, the numbers of chickens infected with pathogens dropped. Chickens with clinical infection were dying from the severity of illness; thus, while they were dying, the populations of clinical infection depleted (B4, B8 and B11).

The SD model of NE and coccidiosis are established upon the main SD model of the broiler house flight simulator (Figure 2.2) (Galarneau et al., 2017) which represents the cyclic process of broiler production within a flock. The main model was incorporated with a grow-out period which began on day 1 to day 42 and a period of down time. However, the CLDs were not a model for simulation, but they illustrated the relationships to be modeled. The stocks and flows of SD model were developed by using the Vensim® (Professional for Window 6.1c, Ventana System, Inc., Harvard, Massachusetts) software, to run the simulations and to test the validity of our initial hypothesis defined in the CLDs.

2.1.3 The stocks and flows

2.1.3.1 The submodels of NE, avian coccidiosis and coinfection

In the submodels, the compartments followed the depiction of above-mentioned CLDs were built up as five populations which made the accumulation of chickens in different stages over time (Figure 2.3and Figure 2.4). Firstly, the population of susceptible chickens was generated by the delivery size of 20,000 birds per flock and separated into two groups of NE-susceptible and coccidia-susceptible chickens (Hein, 1971). Secondly, these susceptible populations gradually became populations incubated with pathogenic *Cl. perfringens* and *Eimeria* spp., under main influences of their infectivity and effective contacts. The contact rate was set as 10 chickens per day (Rhodes and Anderson, 2008) in the same house. Thirdly, under the effects of three fractions made for the subclinical infection, clinical infection and coinfection, chickens effectively infected with either pathogen, were showing minor to severe clinical signs and being divided into two populations, which were subclinical and clinical forms. Clinical infected chickens were dying from it severity, moved towards the coinfection group, or turned into the subclinical group with certain recovery. The starting days (NE: Cooper and Songer, 2010; coccidiosis: Levine, 1961) and infectivity of subclinical and clinical form of NE (Kaldhusdal and Hofshagen, 1992; Lovland et al., 2003) and avian coccidiosis (Williams, 1999; Zhang et al., 2013) were variables input into the fractions. Meanwhile, with the influence of its recovery rate , the reciprocal of infectious period (Helmboldt and Bryant, 1971; Levine, 1961), a proportion of the clinical infected chickens were assumed to turn into subclinically infected which dynamically increased the subclinical infected population. Fourthly, driven by the fatality of NE (Shane et al.,

1985) and coccidiosis (Levine, 1961) as well as the fraction of one pathogen coinfected with the other pathogen, clinical infected chickens were either entered into coinfection or dying from the severity and removed from the flock. Eventually, chickens which were not died were harvested at the end of the grow-out period.

In the submodel of coinfection of NE and coccidiosis (Figure 2.5), the main population was accumulated by four populations of subclinical NE, clinical NE, subclinical coccidiosis and clinical coccidiosis under the influence of the coinfection fractions in their submodels. The proportion thresholds of NE and coccidiosis (Figure 2.3and Figure 2.4) were assumed to be the initial proportions of infected chickens in the population that must exceed a certain number, which depends upon the NE and coccidial infectivity, to cause the occurrence of coinfection (Jeffers, 1974; Shane et al., 1985). This coinfected population was either dying from the severe illness (Shane et al., 1985) or being harvested at the end of the grow-out period.

All chickens dying from NE, coccidiosis or both were removed from the flock. Other predisposing factors, including contact rate, feed composition, anticoccidial vaccine, anticoccidial drugs and antimicrobial drugs, were added to affect the variation of each population. The effect of feed composition with different percentages of corn, NSP and animal protein content was developed in the NE submodel to observe the outcome of varying these content (Olkowski et al., 2006). The stock and flow of the antimicrobial treatment was incorporated in the NE submodel with the NE mortality and an antimicrobial withdrawal period (Shojadoost et al., 2012) under an assumed efficacy which depended on different antimicrobials. In the submodel of coccidiosis, the stock and flow of anticoccidial drugs with coccidial mortality (Zhang et al., 2013) and anticoccidial

withdrawal period (Duquette, 2005) as well as the effect of anticoccidial vaccines (Williams et al., 1999) were incorporated with assumed efficacies which depended on different anticoccidial drugs and vaccines. The exogenous and endogenous variables of the submodels of NE and avian coccidiosis were listed in the Appendix (App.1and App.2).

2.1.3.2 The submodels of average weights

To have a fundamental estimation of the overall performance of the flock, the submodel of average weights varied by different populations in the submodels of NE, avian coccidiosis and coinfection were developed under the basic growth performance of Cobb-500 broiler as hatched (Cobb-vantress, 2015). In this submodel, the percentages of average weight losses of different forms of coccidiosis, NE and coinfection in different groups were input into the submodel. Therefore, not only the average weights of chickens infected with subclinical infection, clinical infection or coinfection were estimated (Figure 2.6) at any point of time, but also the average weights of grow-out chickens which excluded the infected and dead ones was calculated at the same time. The exogenous and endogenous variable of the submodel of average weights were listed in the Appendix (App.1and App.2).

2.2 MODEL VALIDATION

Three types of sensitivity analyses were implemented in the SD model, including the numerical sensitivity, behavioral sensitivity and structural sensitivity (Martinez and Otto, 2002). The numerical sensitivity is to change the numbers of the output of the simulation but not the behavioral pattern. The behavioral sensitivity is to change the

numbers and the behavioral pattern of the output of the simulation. The structural sensitivity is to change the structure which led to the change of output. The process of doing the numerical sensitivity analysis was as follows: (1) Listing the exogenous parameters and relationships which were under investigation of significance; (2) Determining the possible range of selected parameters which included the setting value in the model; (3) Running the model under a full range of different values for that parameter while holding everything else constant (Martinez and Otto, 2002). The numerical sensitivity analysis was applied by using the random uniform distributions with the univariate analysis, the results of sensitivity analysis presented in confidence bounds which represented the possibilities in given simulations. Moreover, the vector distribution with univariate analysis was applied to the scenario analysis which provided the trends of different interventions.

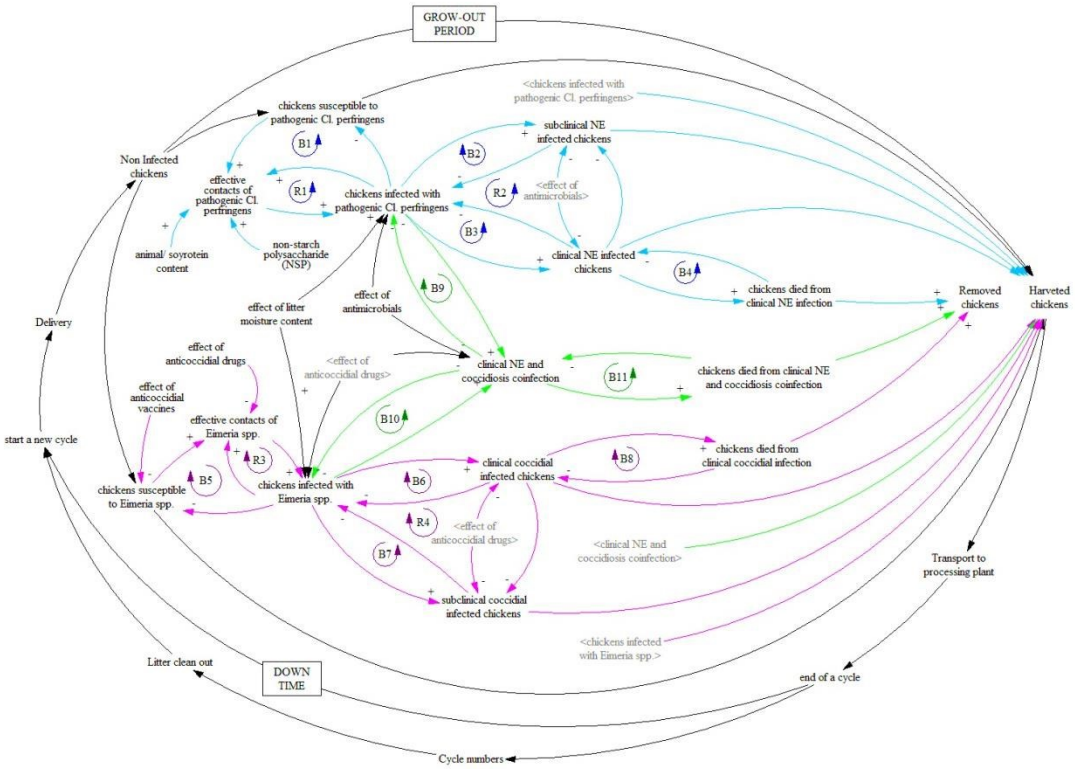

Figure 2.1 The causal loop diagrams of epidemiology of NE and its predisposing factors

The lines in blue, pink and green colors represented the relationships of susceptible, infected and removed chickens in the occurrence of NE (blue lines), avian coccidiosis (pink lines) and coinfection (green lines). The variations of disease epidemiology occurred on the basis of broiler house simulator which presented a cycle of grow-out period regarding broiler production, starting day-old to day 42.

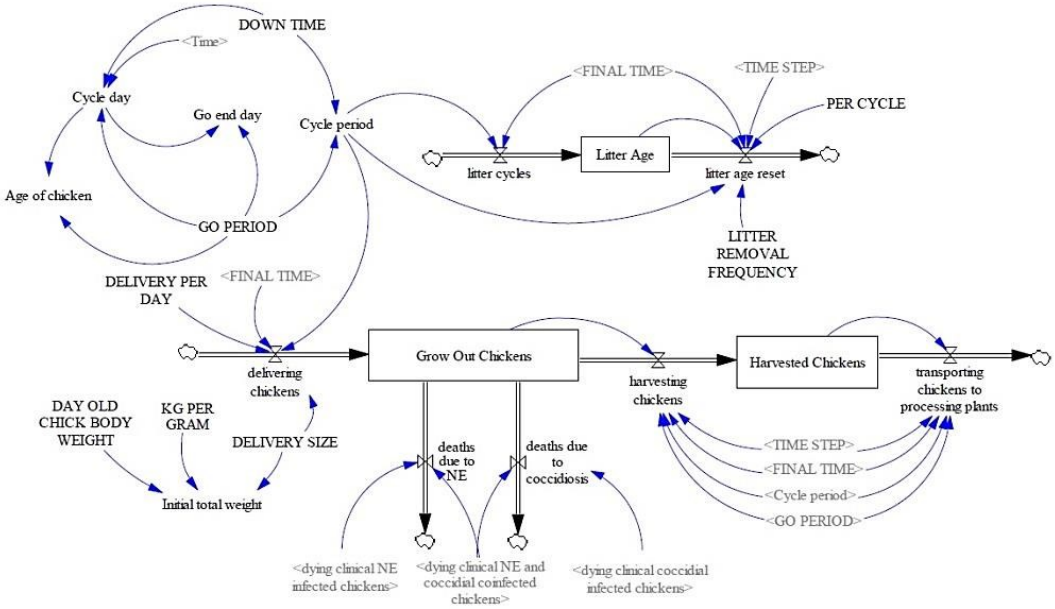

Figure 2.2 The main model

The main model is a broiler house flight simulator which worked as the cyclic process of broiler production within a flock. The grow-out cycle was incorporated with a grow-out period of 42 days and a down time of 14-days.

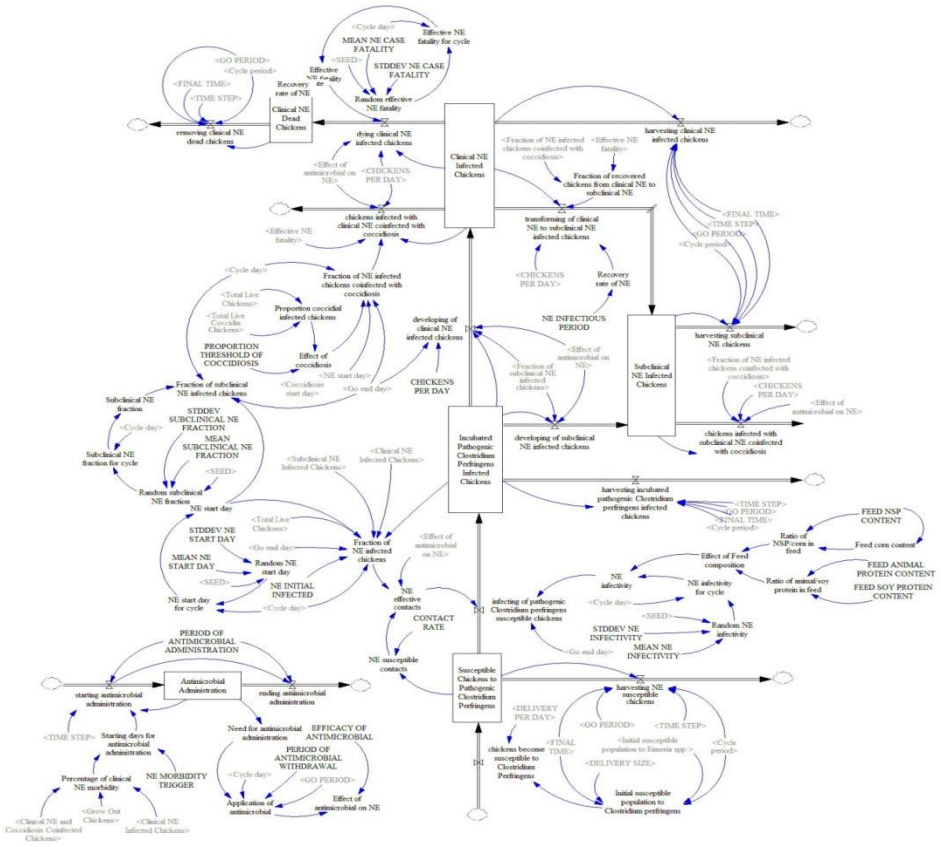

Figure 2.3 The submodel of NE

Firstly, the population of susceptible chickens was assumedly generated by the delivery size flock and separated into two groups of pathogenic *Cl. perfringens*-susceptible and *Eimeria* spp.-susceptible chickens in two submodels. Secondly, the NE-susceptible populations gradually became populations incubated with pathogenic *Cl. perfringens* under main influences of their infectivity and effective contacts. Thirdly, under the effects of three fractions made for the subclinical infection, clinical infection and coinfection, chickens effectively infected with pathogenic *Cl. perfringens* were showing minor to severe clinical signs and divided into three populations. The starting days and infectivity of subclinical and clinical form of NE were variables input into the fractions. Meanwhile, with the influence of its recovery rate or infectious period, a proportion of the clinical infected chickens were assumed to turn into subclinical infection which dynamically increased the subclinical infected population. Fourthly, driven by the fatality of NE, clinical infected chickens were dying from the severity of illness and removed from the flock. Eventually, chickens which were not dying from NE were harvested at the end of the grow-out period.

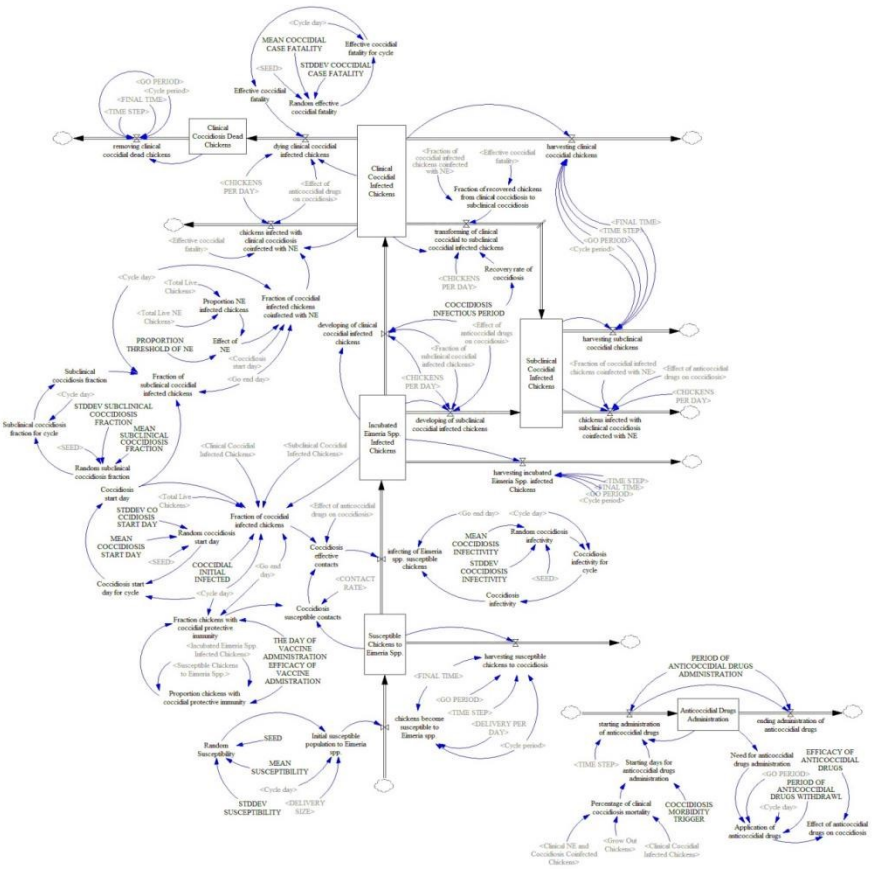

Figure 2.4 The submodel of avian coccidiosis

Firstly, the population of susceptible chickens was assumedly generated by the delivery size flock and separated into two groups of *Eimeria* spp.-susceptible and pathogenic *Cl. perfringens*-susceptible chickens in two submodels. Secondly, the *Eimeria* spp.-susceptible populations gradually became populations incubated with *Eimeria* spp. under main influences of their infectivity and effective contacts. Thirdly, under the effects of three fractions made for the subclinical infection, clinical infection and coinfection, chickens effectively infected with pathogens were showing minor to severe clinical signs and divided into three populations. The starting days and infectivity of subclinical and clinical form of coccidiosis were variables input into the fractions. Meanwhile, with the influence of its recovery rate or infectious period, a proportion of the clinical infected chickens were assumed to turn into subclinical infection which dynamically increased the subclinical infected population. Fourthly, driven by the fatality of coccidiosis, clinical infected chickens were dying from the severity of illness and removed from the flock. Eventually, chickens which were not dying from coccidiosis were harvested at the end of the grow-out period.

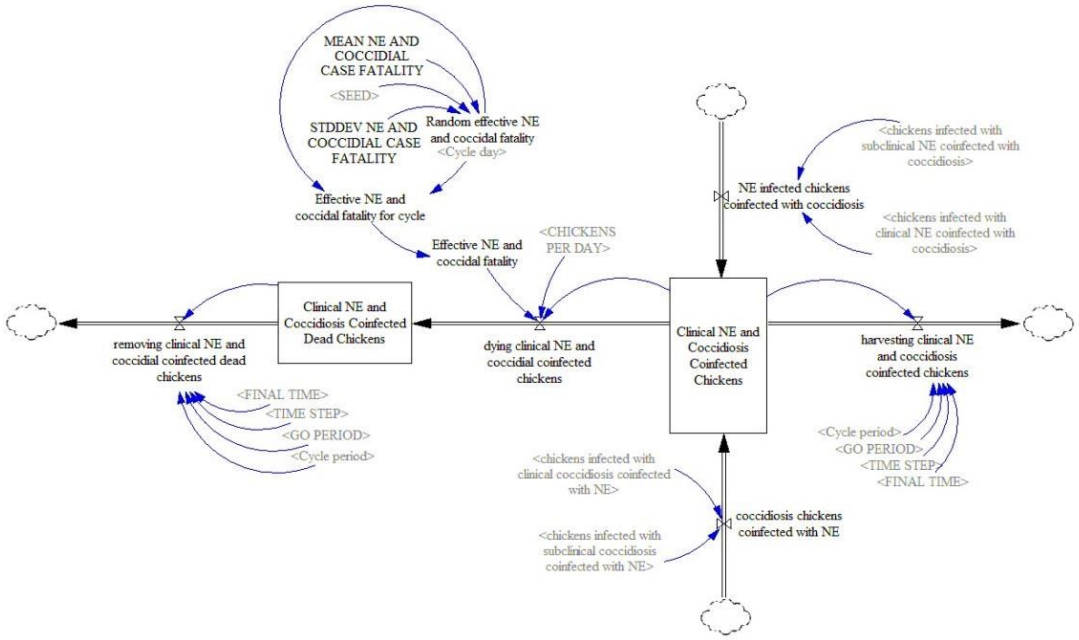

Figure 2.5 The submodel of NE and avian coccidiosis coinfection

The main coinfected population was accumulated by two populations of clinical NE and coccidial infection under the influence of the coinfection fractions in their submodels. This coinfected population was either dying from the illness because of the effects of fatality or being harvested at the end of the grow-out period.

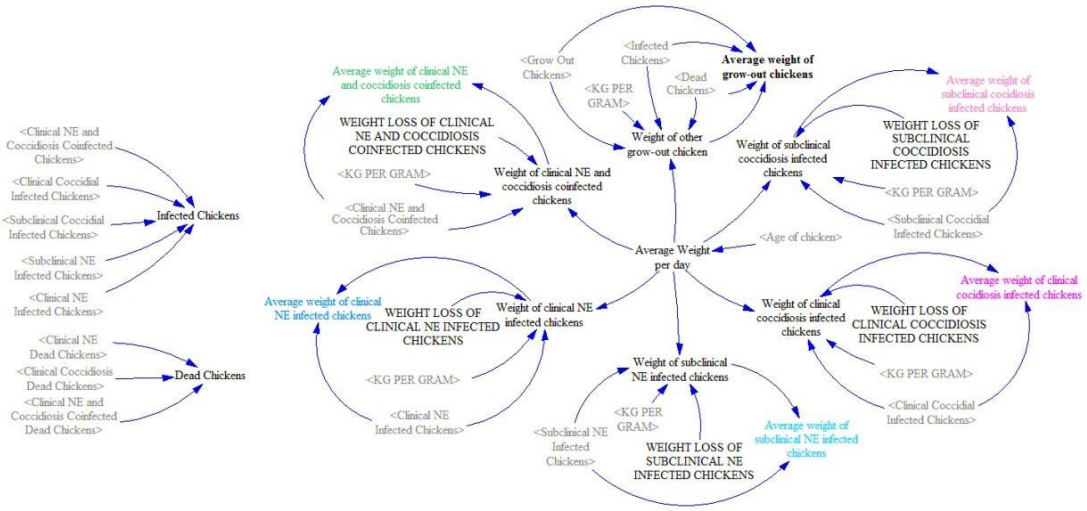

Figure 2.6 The submodel of average weights of chickens infected with NE, avian coccidiosis and coinfection

In this submodel, the average weights of chickens infected with subclinical infection, clinical infection or coinfection were estimated by different weight losses caused by diseases at any point in the grow-out cycle, including at harvest. The average weights of grow-out chickens which excluded the infected and dead chickens were calculated by normal daily average weight simultaneously.

CHAPTER III

RESULTS

3.1 THE SUBMODELS OF DISEASE DYNAMICS

3.1.1 The patterns of disease epidemiology

In the SIR model of NE, avian coccidiosis and coinfection which were developed to obtain better understanding of disease epidemiology, each populations of susceptible, infected during incubation period, clinically infected, subclinically infected, and dead chickens were presented graphically with patterns in the disease progression (Figure 3.1). Under the condition of no antimicrobial administration, in one grow-out cycle, as the clinical coccidiosis occurred in the flock at the age of 2 weeks, the occurrence of clinical NE followed in the next week (the 3rd week) with a coinfection of both diseases which began at the recession of coccidiosis (Figure 3.2). The epidemic curves matched the general observations on the occurrence and relationships of NE and coccidiosis (Williams, 2005). In addition, in the same grow-out cycle, the epidemiological patterns of subclinical infected as well as dead chickens were depicted and followed the trend described in several experimental models (Figure 3.3) (Kaldhusdal and Hofshagen, 1992; Kaldhusdal and Skjerve, 1996; Shane et al., 1985). By setting the duration of the simulation to 340 days and stochastically determining certain exogenous variables by using randomly assigned values from the normal distributions based on designated means and standard deviations (App.1), the variations of disease epidemiology in multiple grow-

out cycles in a year were displayed. The estimate of each population in each grow-out period was calculated and presented within a flow of six cycles (Figure 3.4).

3.1.2 The sensitivity analysis of disease submodels

Selected variables were tested in sensitivity analysis in one grow-out period by using randomly assigned values from the uniform distributions based on designated minimum, and maximum values for each variable (Table 3.1). With 200 simulations, the different confidence boundaries of selected variables were depicted to compare with the baseline output produced by holding all variables at their mean values. The confidence boundaries showed different confidence intervals of chickens in different populations over time (50% confidence, yellow area; 75% confidence, green area; 95 % confidence, blue area; 100% confidence, grey area). All simulations were enclosed in 0 to 100 % confidence boundary. By eliminating the lower and top 5 runs, the 95% confidence boundary was formed, and the following eliminations established other smaller confidence boundaries with different percentage which might be translated into tolerance intervals (Ford and Flynn, 2005). As the time went from day 1 to day 42, daily confidence intervals of chickens in each population cumulated as a continuous graph of belts or bells. Each confidence interval gave us a range of plausible values for the numbers of chickens in different populations that varied by time. In one grow-out period, the mean values of chickens infected with subclinical and clinical NE, subclinical and clinical coccidiosis and coinfection had similar patterns as the baseline settings for the variables that were tested in the sensitivity analysis. The explanatory variables which belonged to management practice were tested, including contact rate (Figure 3.5), feed animal protein content (Figure 3.6), feed soy protein content (Figure 3.7), feed NSP

content (Figure 3.8), the efficacies of anticoccidial vaccine (Figure 3.9), anticoccidial drug (Figure 3.10) and antimicrobial (Figure 3.11). Other variables tested in the Table 3.1 were mean subclinical NE infectivity, mean subclinical coccidial infectivity, mean clinical NE infectivity, mean clinical coccidial infectivity, and mean susceptibility. These explanatory variables belonged to the intrinsic ability of host infection. The results of the sensitivity analyses demonstrated that the sensitivity level of responding variables was changed when selected explanatory variables varied randomly about their distributions.

3.1.3 The scenario analysis of disease submodels

Different scenarios were evaluated by running simulations of the model in which each of the variables tested in the sensitivity analysis were incrementally changed through a range of values to determine how these changes might affect the epidemiology of NE and coccidiosis in one grow-out period (Table 3.2), Using vector distributions, these scenarios of sensitivity analysis, provided important insights which corresponded with the observations in literature. Firstly, a higher contact rate increased the incidence of subclinical and clinical coccidiosis, subclinical and clinical NE, and concurrent infection (Figure 3.12). Secondly, decreasing the percentage of animal protein in feed decreased the incidence of subclinical coccidiosis after coinfection occurred, clinical NE and coinfection, but it had no effect on subclinical coccidiosis before coinfection occurred and clinical coccidiosis (Figure 3.13). Thirdly, adding higher content of soy protein in feed inversely decreased the incidence of subclinical NE, clinical NE and coinfection but had no effect on coccidiosis (Figure 3.14). Regarding the common application of soy protein content ranges from 10 to 50%, a further analysis was processed to see the variations (Figure 3.15). Fourthly, adding a higher NSP content in feed increased the

incidence of subclinical coccidiosis after coinfection occurred, clinical NE, and coinfection, but it had no effect on subclinical coccidiosis before coinfection occurred and clinical coccidiosis (Figure 3.16). Regarding the common application of NSP content ranges from 10 to 50%, a further analysis was conducted to see the variations (Figure 3.17). Fifthly, the incidence of subclinical and clinical coccidiosis decreased when the efficacy of anticoccidial vaccine increased, but there were slightly inverse effects on subclinical NE, clinical NE and coinfection (Figure 3.18). Sixthly, the incidence of subclinical and clinical coccidiosis decreased when the efficacy of anticoccidial drug increased; besides, there was a comparatively little effect on subclinical NE, clinical NE and coinfection (Figure 3.19). The last result of the efficacy of antimicrobial showed that the incidence of subclinical coccidiosis after coinfection occurred, subclinical NE, clinical NE and coinfection decreased when the efficacy of antimicrobial increased, but there was no effect on subclinical coccidiosis before coinfection occurred and clinical coccidiosis (Figure 3.20).

3.2 THE SUBMODELS OF AVERAGE WEIGHTS

3.2.1 The patterns of growth performance

In this submodel, the variation of average weights of infected population with clinical signs, without clinical signs, and with death were calculated in one cycle or multiple cycles. In one grow-out period, the subclinical coccidial infected chickens were gaining more weight than subclinical NE infected chickens because of less severe weight loss due to coccidial infection; in addition, the average weights of chickens infected with clinical NE, coccidiosis and coinfection accumulated differently representing variations in population sizes and the effect of disease conditions on individual weight loss (Figure

3.21). In one grow-out period, the comparative patterns of average weights in chickens without infection, infected with subclinical, clinical and concurrent infection were estimated (Figure 3.22). The average weights of subclinical infected, clinical infected, concurrent infected and non-infected chickens were calculated (Table 3.3).

Table 3.1 The values of selected variable used for random uniform distribution of the sensitivity analysis

Variable		Baseline value	Minimum	Maximum
1	CONTACT RATE	10.00	5	15
2	FEED ANIMAL PROTEIN CONTENT	0.03	0.01	0.10
3	FEED SOY PROTEIN CONTENT	0.24	0.10	0.90
4	FEED NSP CONTENT	0.60	0.10	0.90
5	MEAN COCCIDIOSIS INFECTIVITY	0.41	0.10	0.90
6	MEAN NE INFECTIVITY	0.46	0.10	0.90
7	MEAN SUBCLINICAL COCCIDIOSIS INFECTIVITY	0.26	0.10	0.90
8	MEAN SUBCLINICAL NE INFECTIVITY	0.25	0.10	0.90
9	MEAN SUSCEPTIBILITY	0.73	0.10	0.90
10	EFFICACY OF VACCINE ADMISTRATION	0.10	0.10	0.90
11	EFFICACY OF ANTICOCCIDIAL DRUG	0.10	0.10	0.90
12	EFFICACY OF ANTIMICROBIAL	0.10	0.10	0.90

Simulations= 200; univariate analysis; one grow-out period
For each variable that was the subject of a sensitivity analysis, the other exogenous variables were held constant without variation except for three variables (10, 11 and 12) which were always stochastically determined.

Table 3.2 The values of selected variable used for vector distribution of the scenario analysis

Variable		Baseline value	Minimum	Maximum	Increment
1	CONTACT RATE	10.00	5	15	2.50
2	FEED ANIMAL PROTEIN CONTENT	0.03	0.01	0.10	0.02
3	FEED SOY PROTEIN CONTENT	0.24	0.10	0.50	0.20
4	FEED NSP CONTENT	0.60	0.10	0.50	0.20
5	EFFICACY OF VACCINE ADMISTRATION	0.10	0.10	0.90	0.20
6	EFFICACY OF ANTICOCCIDIAL DRUG	0.10	0.10	0.90	0.20
7	EFFICACY OF ANTIMICROBIAL	0.10	0.10	0.90	0.20

The simulation depends on the increment of each variable; univariate analysis; one grow-out period

Table 3.3 The average weights of subclinical, clinical, concurrent infected chickens and non-infected chickens at harvest

Population	Subclinical NE	Clinical NE	Subclinical avian coccidiosis	Clinical avian coccidiosis	Coinfection	Non-infection
Average weight (Ib.)	5.54	5.35	5.67	4.04	4.41	6.30
Average weight (Kg)	2.51	2.43	2.57	1.83	2.00	2.86

In one grow-out cycle, the average weights of different populations estimated by this submodel were similar to the performance in the real system. The weights of non-infected chickens averaged 6.3 lb. which reached the marketing weight, while the weights of infected chickens averaged 5.0 lb. (One kilogram equals to 2.205 pounds).

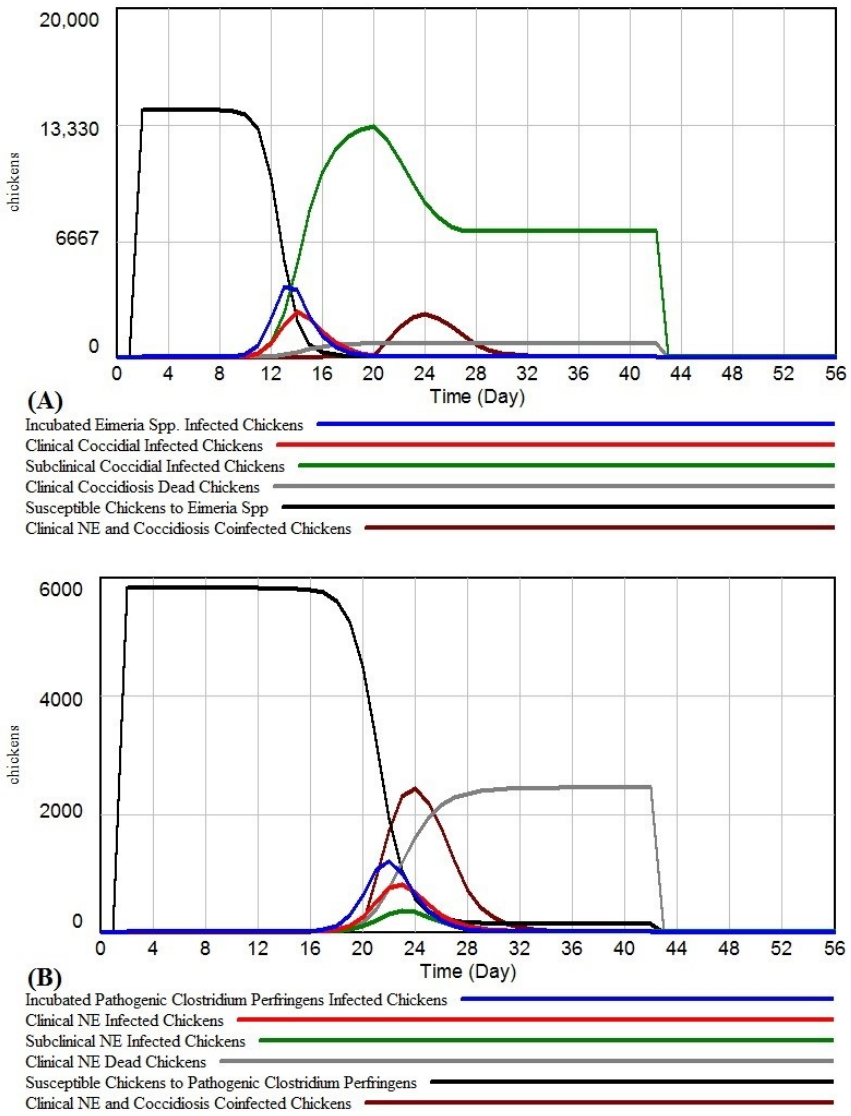

Figure 3.1 The SIR model of NE and coccidiosis in one grow-out cycle

In one grow-out cycle, each population of susceptible, infected during incubation period, clinically infected, subclinically infected, and dead chickens were presented graphically which showed the patterns in disease progression. Clinical coccidiosis was assumed to occur when the chickens were at the age of 14 days (A). Clinical NE was assumed to occur when the chickens were at the age of 22 days (B). The coinfection of NE and coccidiosis was assumed to occur when the chickens were at the age of 24 days (brown line in A and B). The increasing population of clinical NE and coccidiosis coinfection posed an obvious dropdown in the population of subclinical coccidiosis (green line in A).

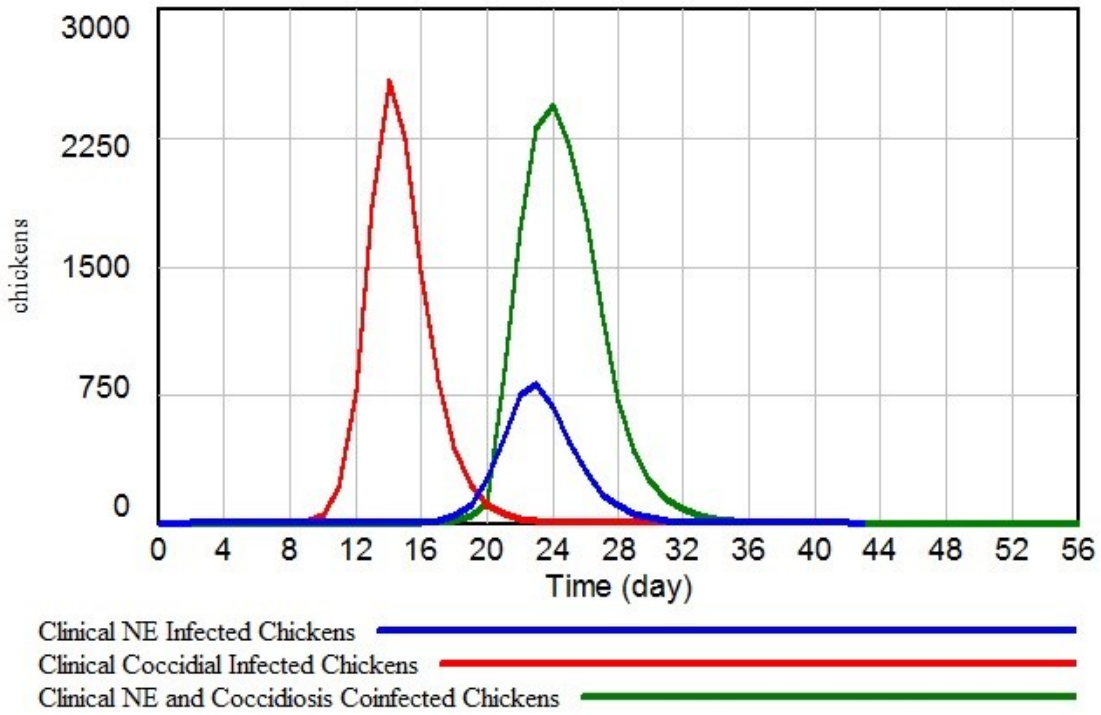

Figure 3.2 The epidemic curves of clinical NE, coccidiosis and coinfection in one grow-out cycle

In one grow-out cycle, as clinical coccidiosis occurred in the flock at the age of 2 weeks, the occurrence of clinical NE followed in the next week (the 3rd week) with a coinfection of both diseases which began at the recession of coccidiosis. The occurrence of clinical NE and coccidiosis caused an epidemic of coinfection.

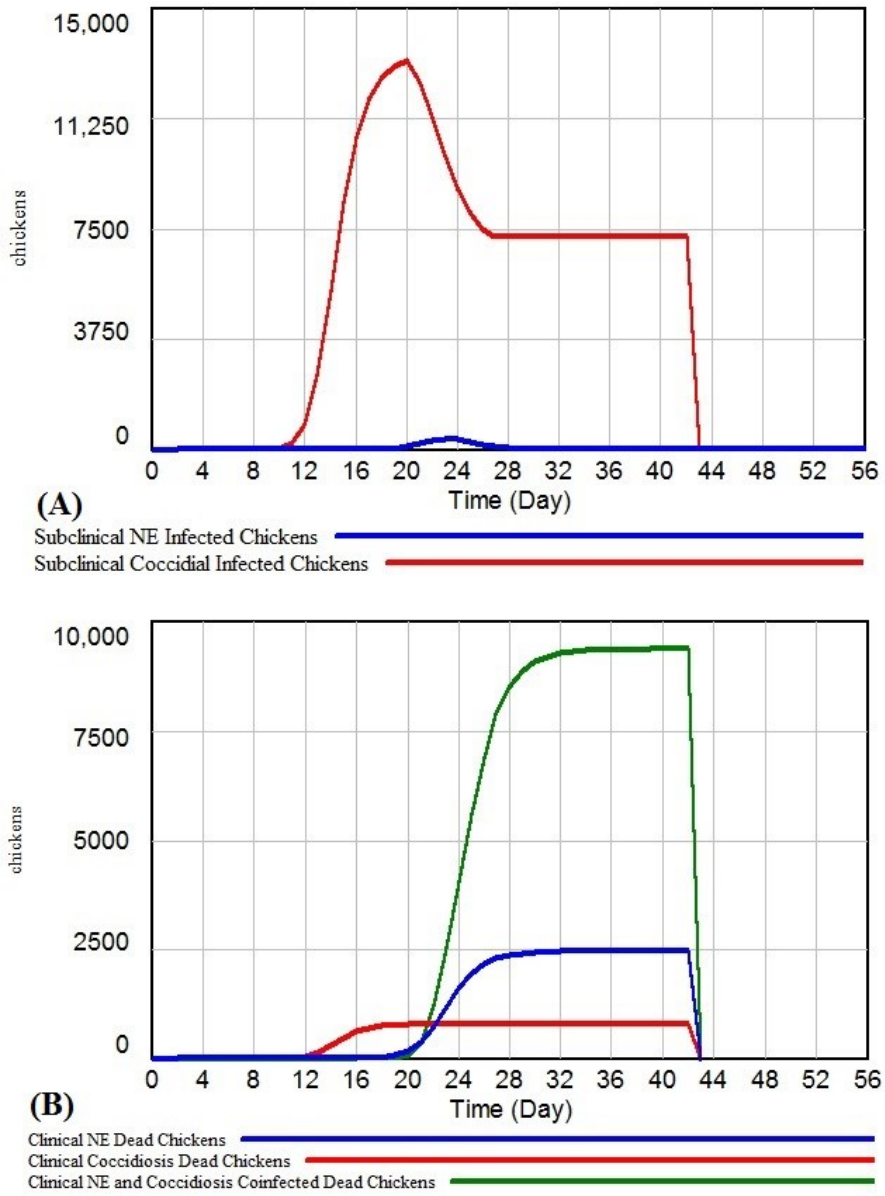

Figure 3.3 The epidemic curves of chickens infected with subclinical NE and coccidiosis and chickens dying from clinical NE and coccidiosis in one grow-out cycle

In one grow-out cycle, chickens infected with subclinical NE had accumulated later and fewer than those with subclinical coccidiosis (A); meanwhile, under the effect of non-antimicrobials, chickens dying from clinical form of NE, coccidiosis and coinfection (B) were accumulated by different starting days.

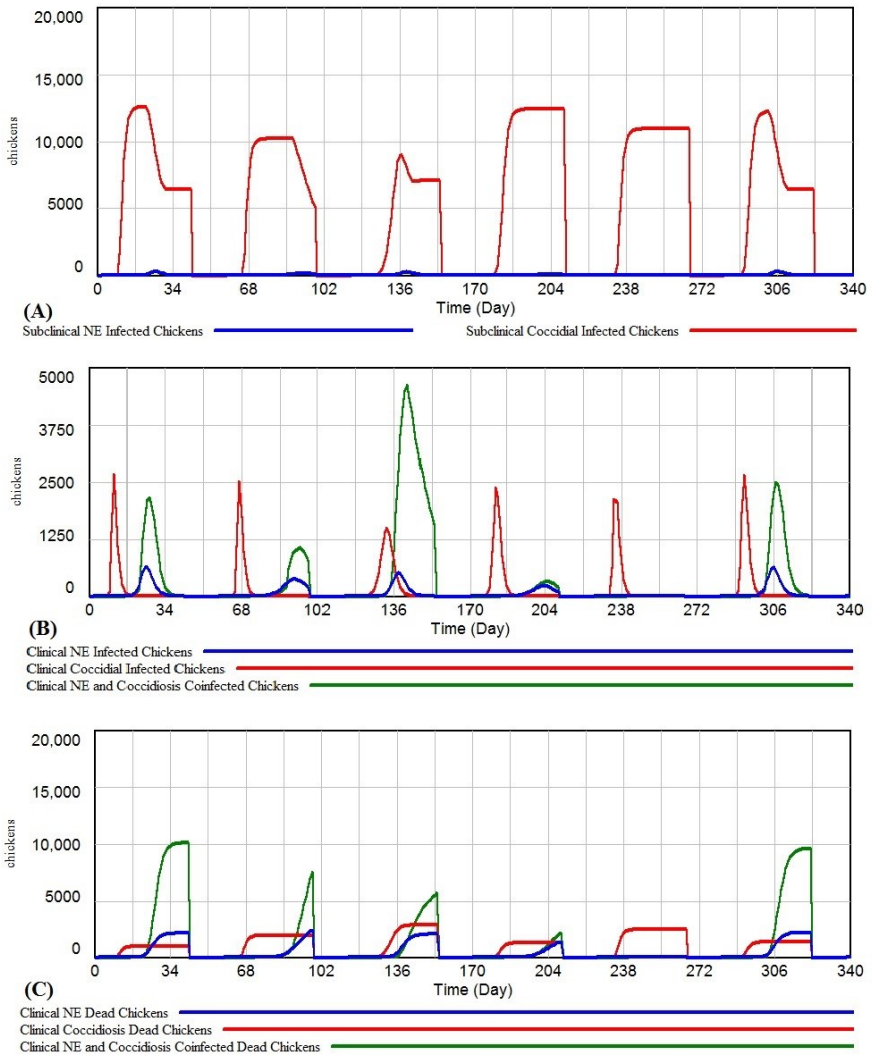

Figure 3.4 The epidemiological patterns of populations of subclinical NE and coccidiosis (A); populations of clinical NE, coccidiosis and coinfection (B); populations dying from clinical form of NE, coccidiosis and coinfection (C) in multiple grow-out cycles

By setting the duration of the simulation to 340 days and stochastically determining certain exogenous variables by using randomly assigned values from the normal distributions based on designated means and standard deviations (App.1), the yearly variations of disease epidemiology in multiple grow-out cycles were displayed. The estimate of each population in each grow-out cycle was calculated and presented in a flow of six cycles. The patterns displayed a trend that concurrent infection of clinical NE and coccidiosis might not occur in every grow-out period.

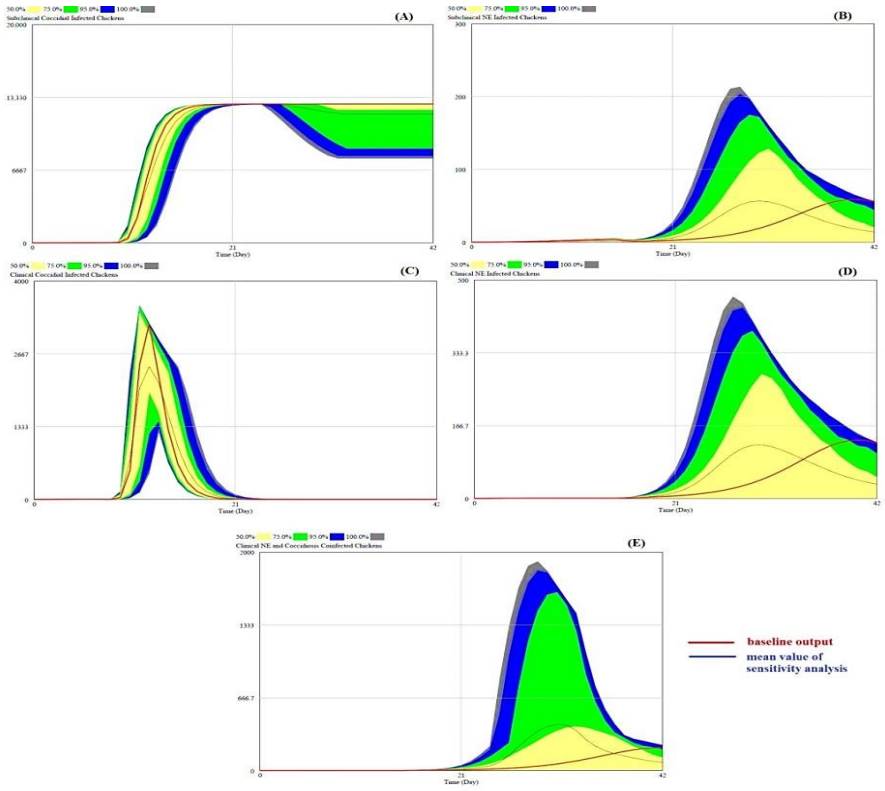

Figure 3.5 The sensitivity analysis of the contact rate applied to the populations of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

1) In one grow-out period, the patterns of mean value and baseline value (10 chickens per day) of chickens infected with subclinical coccidiosis (A), subclinical NE (B), clinical coccidiosis (C), clinical NE (D) and coinfection (E) were showed when the different values (from 5 to 15) of contact rate were randomly assigned. (X axis: days, Y axis: the number of chickens)
2) This figure provided the confidence bounds of all values based on the contact rate when it varied randomly about its uniform distribution with 200 simulations. The confidence boundaries showed different confidence intervals of chickens in different populations over time (50% confidence, yellow area; 75% confidence, green area; 95 % confidence, blue area; 100% confidence, grey area). All simulations were enclosed in 0 to 100 % confidence boundary. By eliminating the lower and top 5 runs, the 95% confidence boundary was formed, and the following eliminations established other smaller confidence boundaries with different percentage which might be translated into tolerance intervals. As the time went from day 1 to day 42, daily confidence intervals of chickens in each population cumulated as a continuous graph of belts or bells. Each confidence interval gave us a range of plausible values for the number of chickens in different population varied by time.

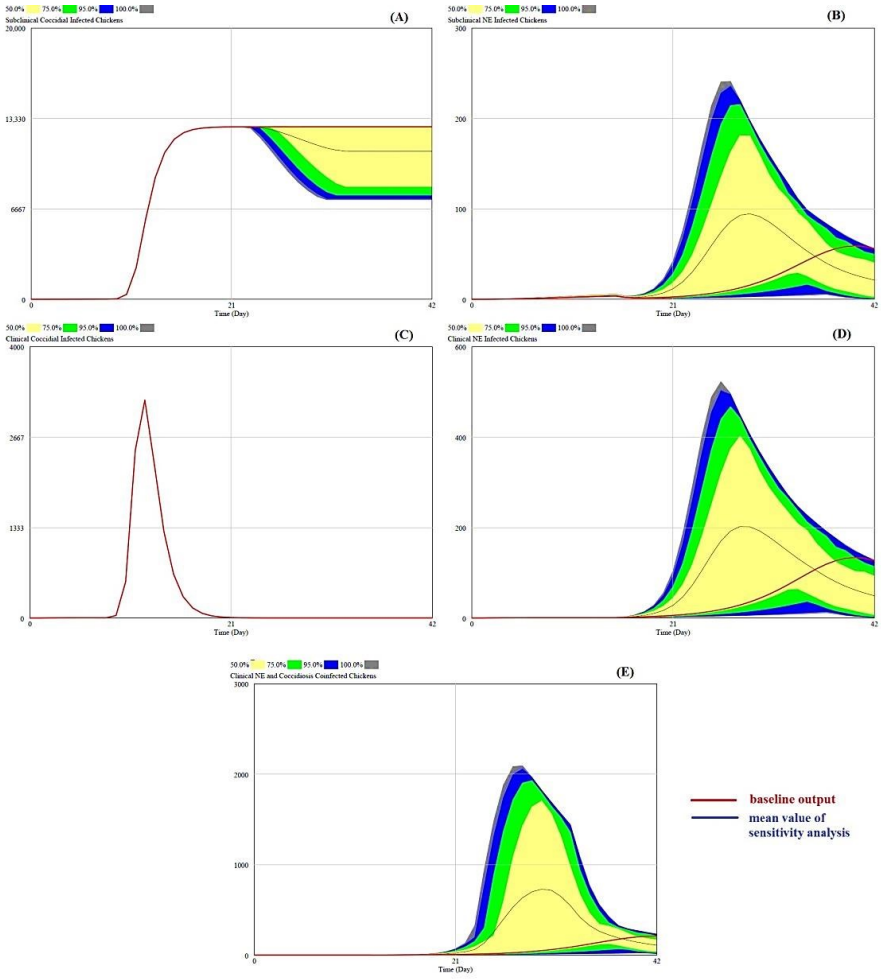

Figure 3.6 The sensitivity analysis of the feed animal protein content applied to the populations of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

1) In one grow-out period, the patterns of mean value and baseline value (3%) of chickens infected with subclinical coccidiosis (A), subclinical NE (B), clinical coccidiosis (C), clinical NE (D) and coinfection (E) were presented when the different values (from 1% to 10%) of feed animal protein content were randomly given. (X axis: days, Y axis: the number of chickens)
2) In this figure, the feed animal protein content showed no effect on subclinical and clinical coccidial infected chickens (A and C). The variation in the population of subclinical coccidiosis (A) after 21st day presented a decreasing population driven by the increasing population of coinfection.

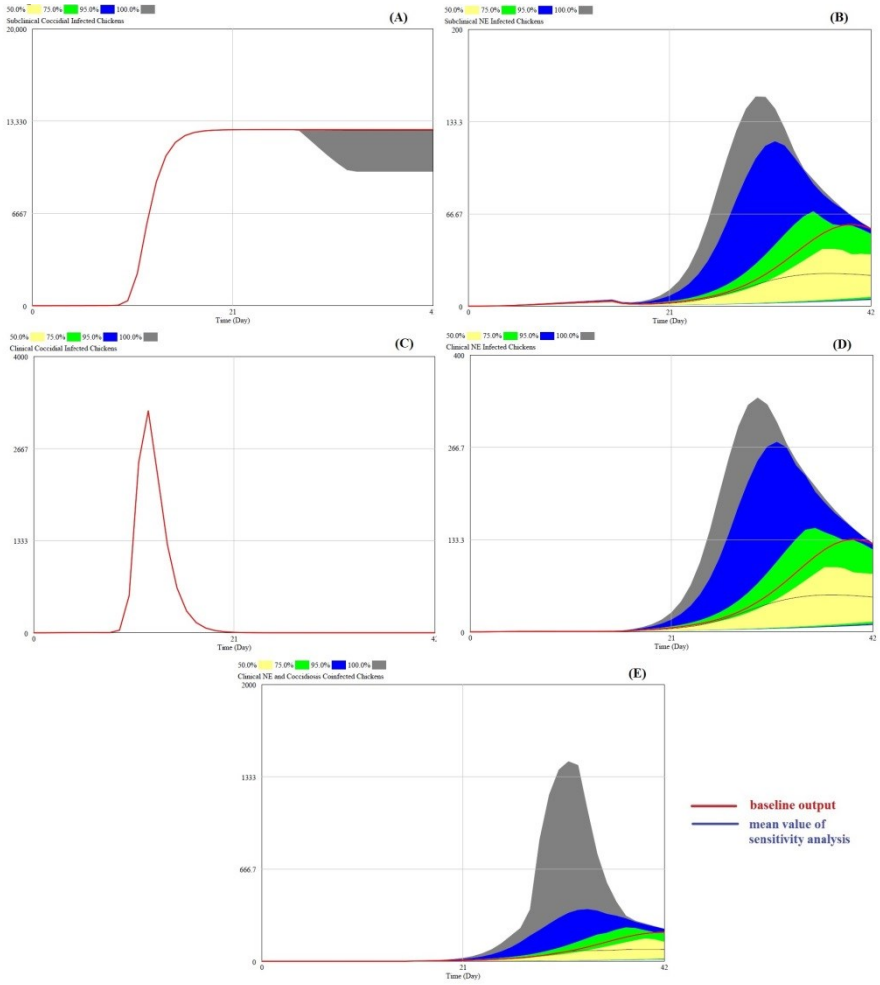

Figure 3.7 The sensitivity analysis of the content of feed soy protein content applied to the populations of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

1) In one grow-out period, the patterns of mean values and baseline value (24%) of chickens infected with subclinical coccidiosis (A), subclinical NE (B), clinical coccidiosis (C), clinical NE (D) and coinfection (E) were showed when the different values (from 10% to 90%) of the content of feed soy protein content were randomly given. (X axis: days, Y axis: the number of chickens)
2) In this figure, the feed soy protein content showed no effect on subclinical and clinical coccidial infected chickens (A and C), and gave less influence on subclinical and clinical NE infected chickens (B and D), and coinfected chickens (E).

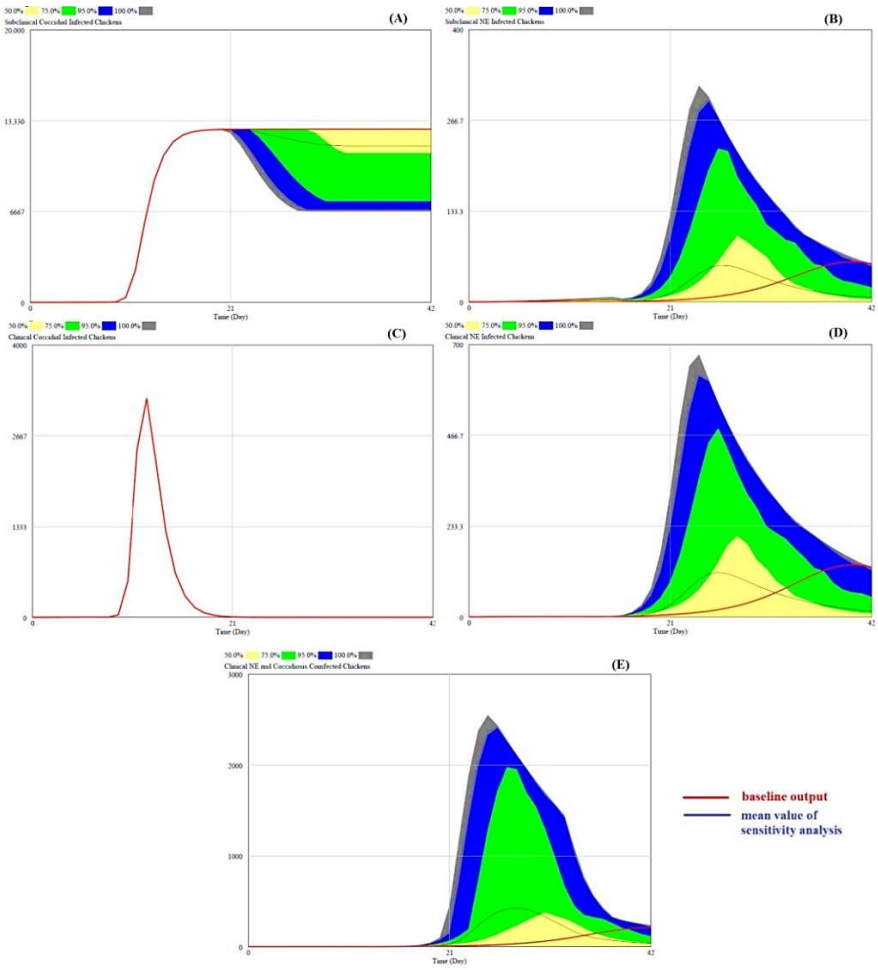

Figure 3.8 The sensitivity analysis of the feed NSP content applied to the populations of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

1) In one grow-out period, the patterns of mean values and baseline value (60%) of chickens infected with subclinical coccidiosis (A), subclinical NE (B), clinical coccidiosis (C), clinical NE (D) and coinfection (E) were presented when the different values (from 10% to 90%) of feed NSP content were randomly given. (X axis: days, Y axis: the number of chickens)
2) In this figure, the feed NSP content showed no effect on coccidial infected chickens (A and C), but significantly affected the NE infected (B and D) and coinfected chickens (E). The variation in the population of subclinical coccidiosis (A) after 21st day presented a decreasing population driven by the increasing population of coinfection.

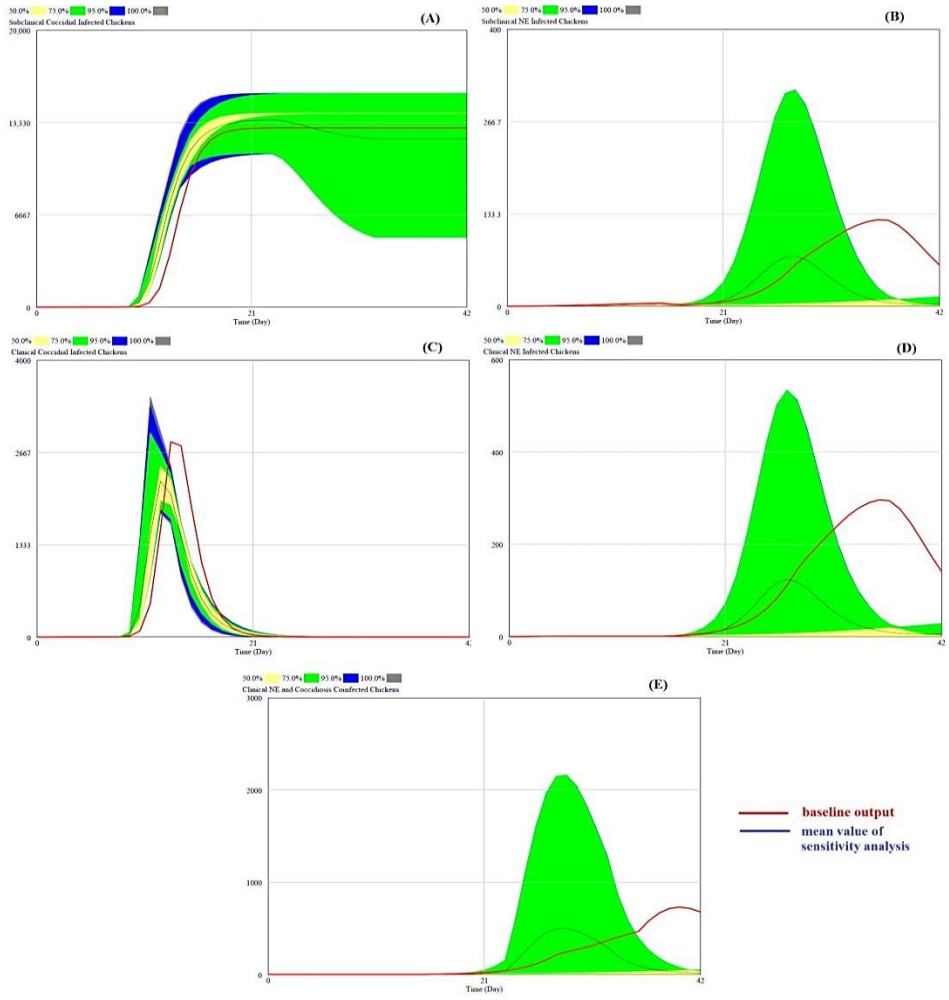

Figure 3.9 The sensitivity analysis of the efficacy of anticoccidial vaccine applied to the populations of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

1) In one grow-out period, the patterns of mean values and baseline value (10%) of chickens infected with subclinical coccidiosis (A), subclinical NE (B), clinical coccidiosis (C), clinical NE (D) and coinfection (E) were presented when the different values (from 10% to 90%) of the efficacy of anticoccidial vaccine were randomly given. (X axis: days, Y axis: the number of chickens)
2) This figure showed that the efficacy of anticoccidial vaccine had more influence on subclinical and clinical coccidial infected chickens (A and C) compared with chickens with subclinical and clinical NE infected chickens (B and D) as well as coinfected chickens (E).

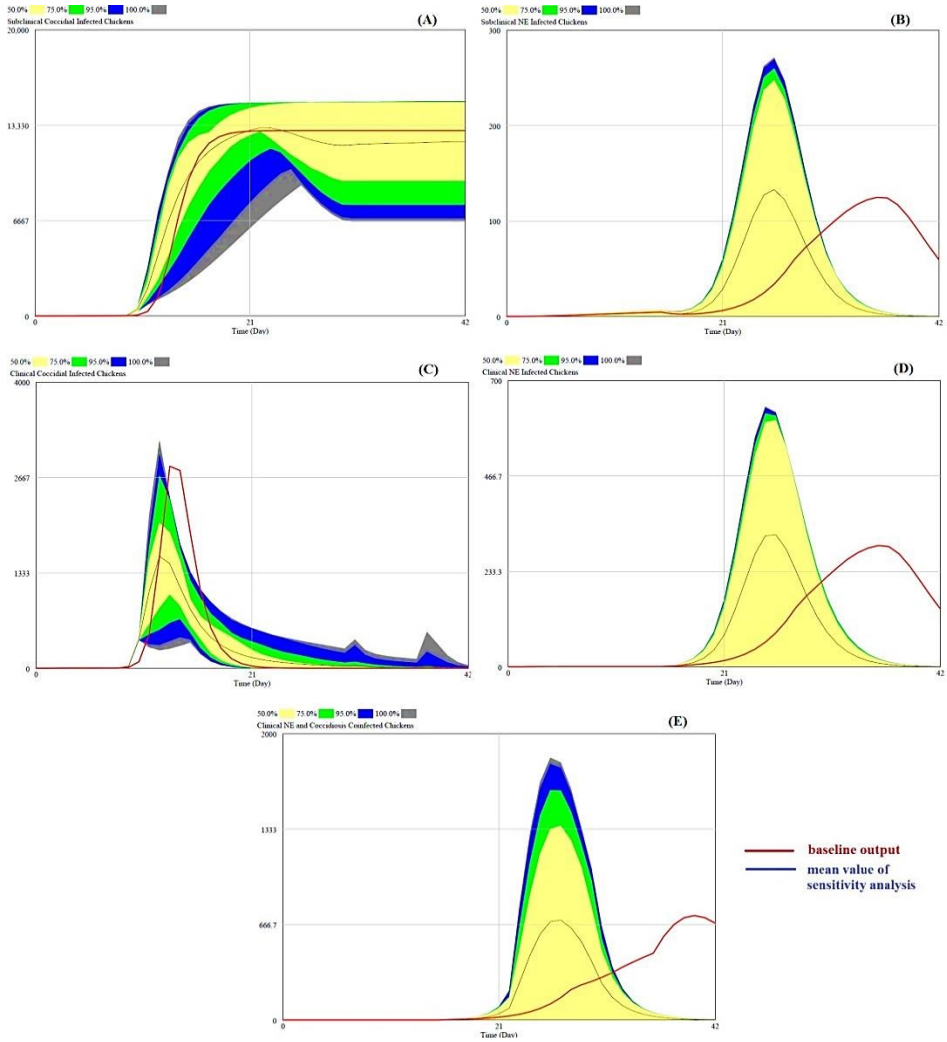

Figure 3.10 The sensitivity analysis of the efficacy of anticoccidial drug applied to the populations of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

1) In one grow-out period, the patterns of mean values and baseline value (10%) of chickens infected with subclinical coccidiosis (A), subclinical NE (B), clinical coccidiosis (C), clinical NE (D) and coinfection (E) were presented when the different values (from 10% to 90%) of the efficacy of anticoccidial drug were randomly given. (X axis: days, Y axis: the number of chickens)
2) This figure presented the anticoccidial drug had effects on chickens infected with subclinical coccidiosis (A), subclinical NE (B), clinical coccidiosis (C), clinical NE (D) and coinfection (E), especially coccidiosis.

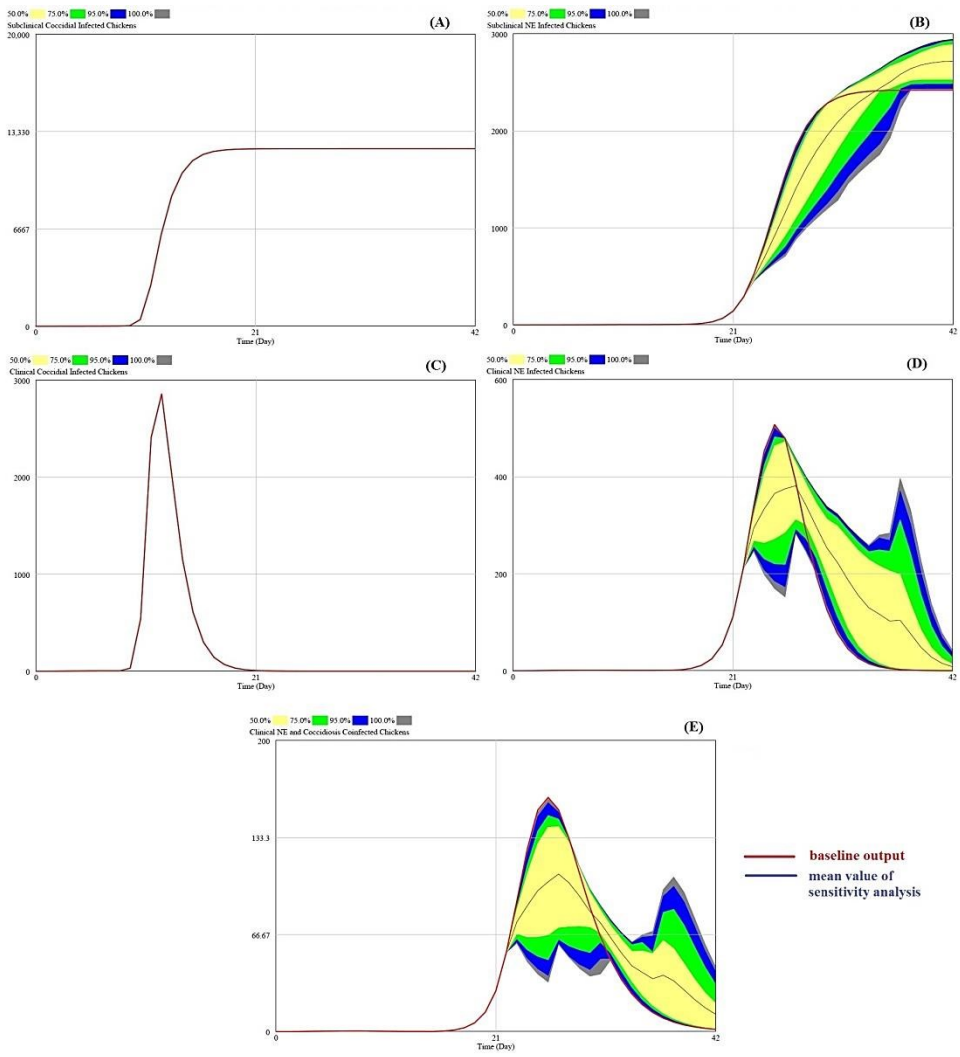

Figure 3.11 The sensitivity analysis of the efficacy of antimicrobial applied to the populations of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

1) In one grow-out period, the patterns of mean values and baseline value (10%) of chickens infected with subclinical coccidiosis (A), subclinical NE (B), clinical coccidiosis (C), clinical NE (D) and coinfection (E) were presented when the different values (from 10% to 90%) of the efficacy of antimicrobial were randomly given. (X axis: days, Y axis: the number of chickens)
2) This figure showed the antimicrobial mainly had effects on subclinical and clinical NE infected chickens (B and D) and coinfected chickens (E).

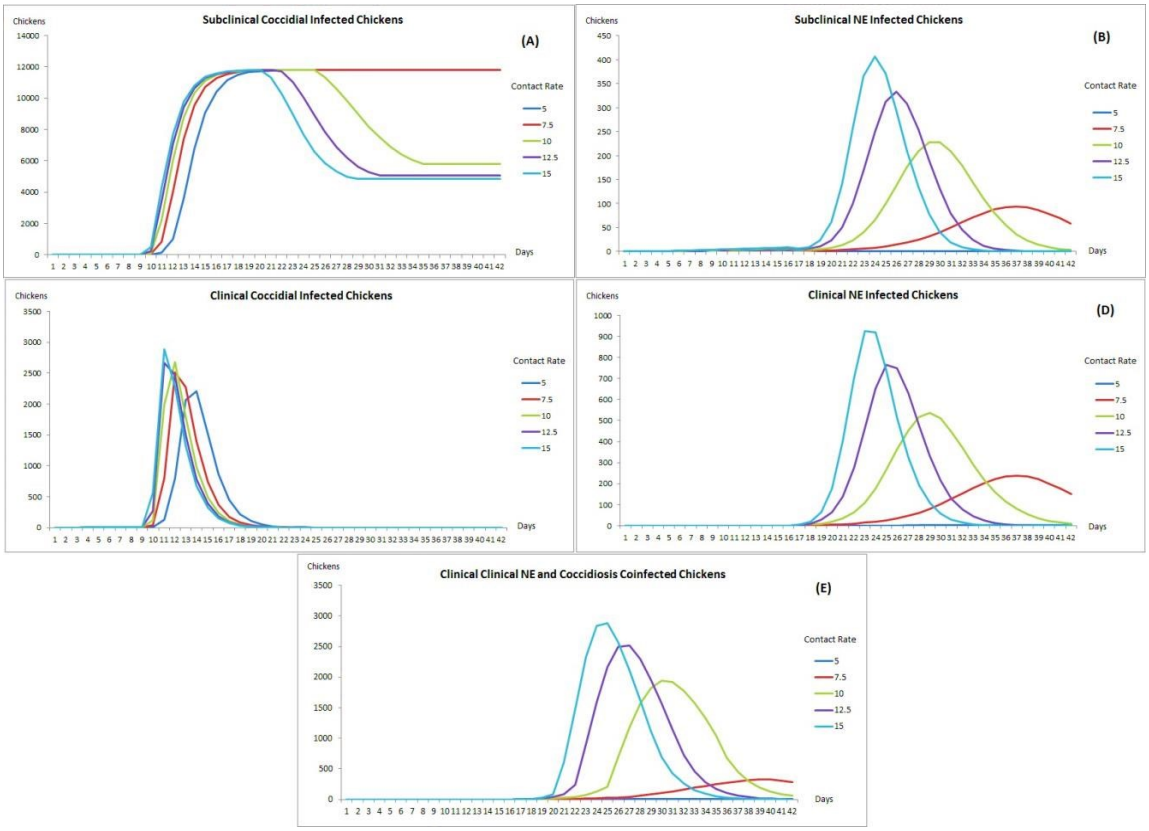

Figure 3.12 The scenario analysis of the contact rate and the incidence of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

Within a set of numbers ranging from 5 to 15 (the setting of contact rate was 10 chickens/ day), the incidence of subclinical coccidiosis (A), subclinical NE (B), clinical coccidiosis (C), clinical NE (D), and coinfection (E) increased when the contact rate increased. After coinfection occurred, the dropping numbers of subclinical coccidial infected chickens were driven by coinfected chickens when the contact rate increased. (X axis: days, Y axis: the number of chickens)

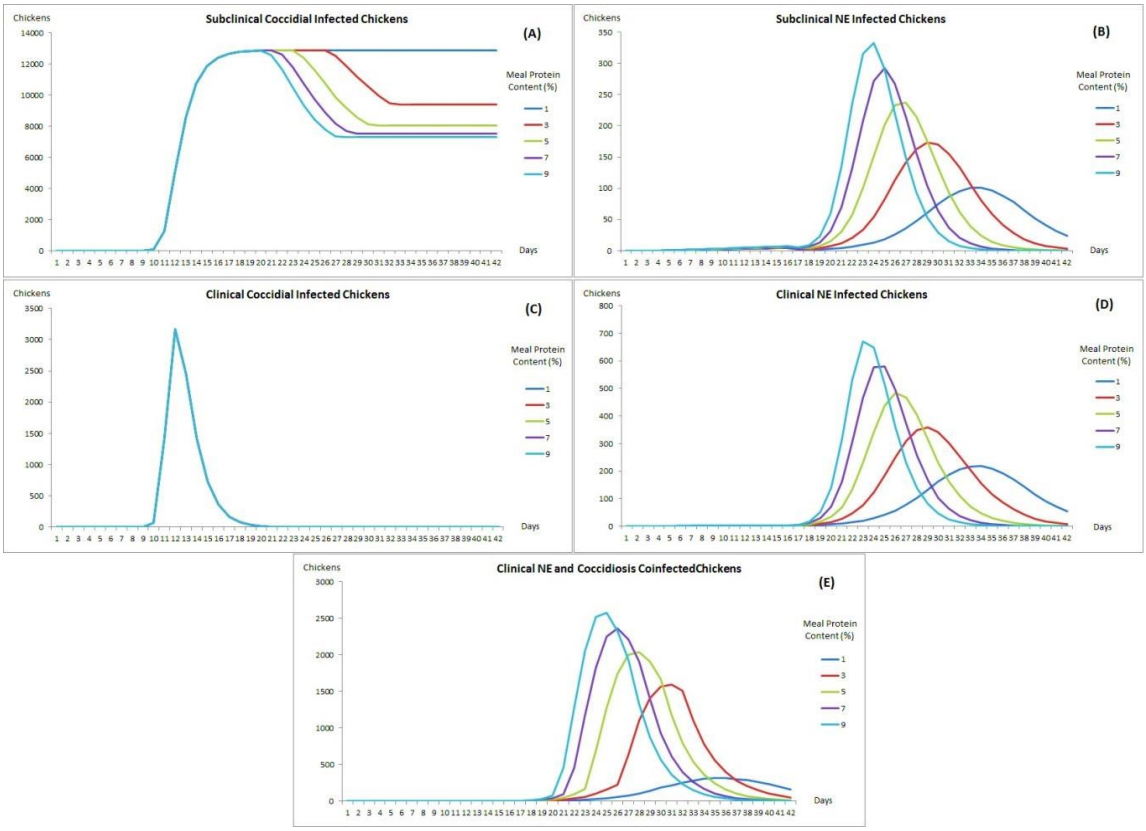

Figure 3.13 The scenario analysis of the animal protein content in feed and the incidence of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

Within a set of numbers ranging from 1% to 9% (the setting of feed animal protein content was 3 %), the incidence of subclinical coccidiosis (A) after coinfection occurred, subclinical NE (B), clinical NE (D) and coinfection (E) decreased when the feed animal protein content decreased, but there was no effect on subclinical coccidiosis (A) before coinfection occurred, and clinical coccidiosis (C). After coinfection occurred, the dropping numbers of subclinical coccidial infected chickens were driven by coinfected chickens when the animal protein content decreased. (X axis: days, Y axis: the number of chickens)

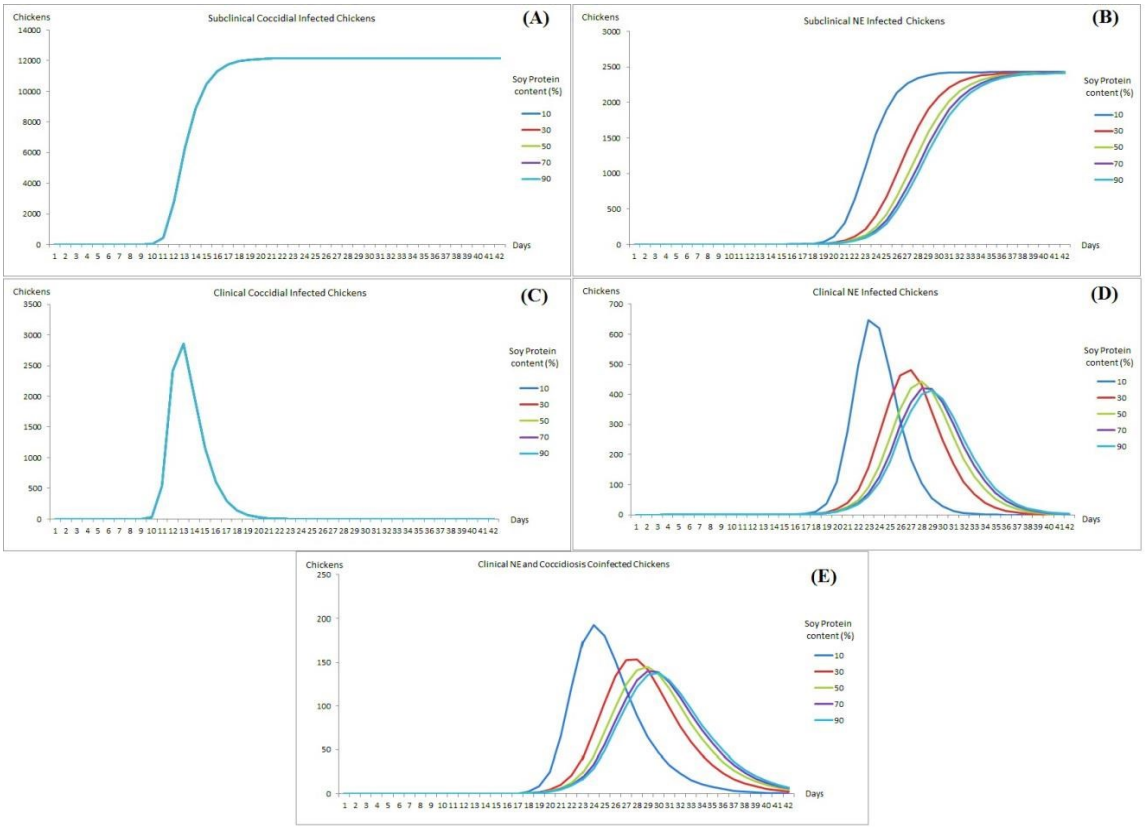

Figure 3.14 The scenario analysis of the feed soy protein content (10% to 90%) and the incidence of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

Within a set of numbers ranging from 10% to 90% (the setting of feed soy protein content was 24 %), the incidence of subclinical NE (B), clinical NE (D) and coinfection (E) decreased when the feed soy protein content increased, but there was no effect on subclinical coccidiosis (A) and clinical coccidiosis (C). (X axis: days, Y axis: the number of chickens)

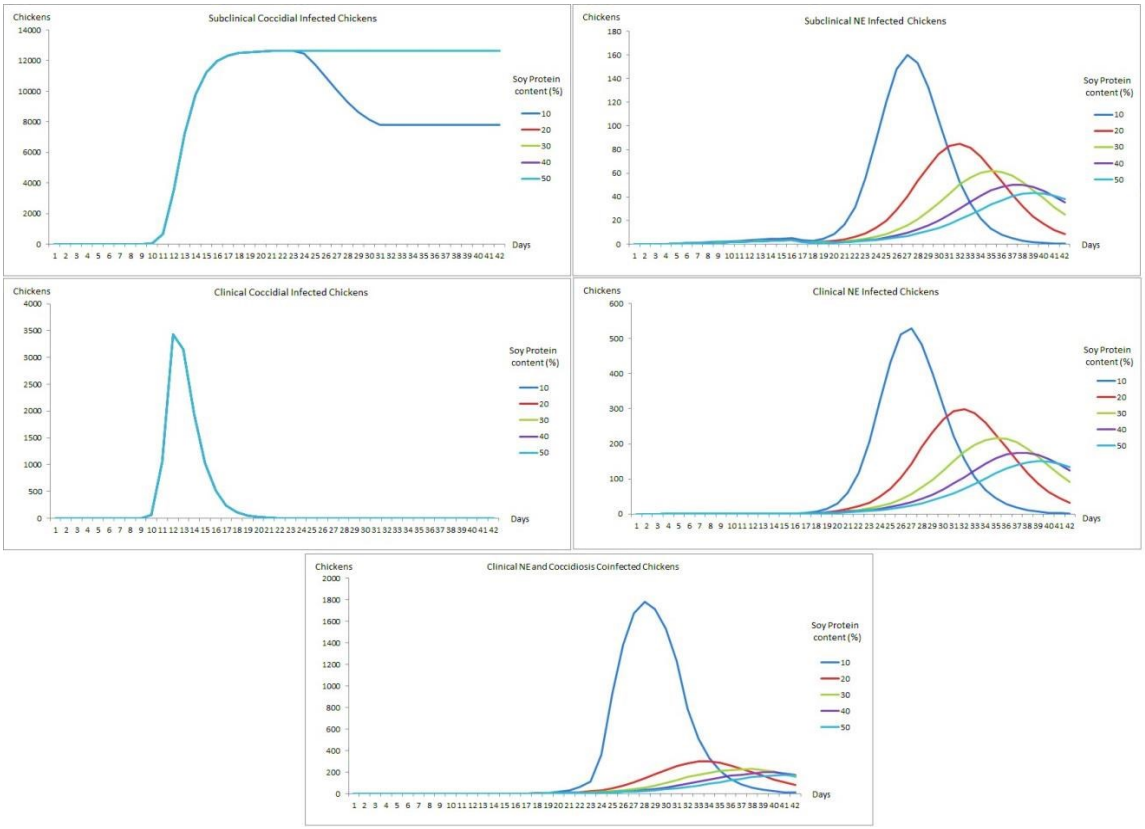

Figure 3.15 The scenario analysis of the feed soy protein content (10% to 50%) and the incidence of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

Regarding the common application of soy protein content ranges from 10 to 50%, a further analysis was processed to see the variations. (X axis: days, Y axis: the number of chickens)

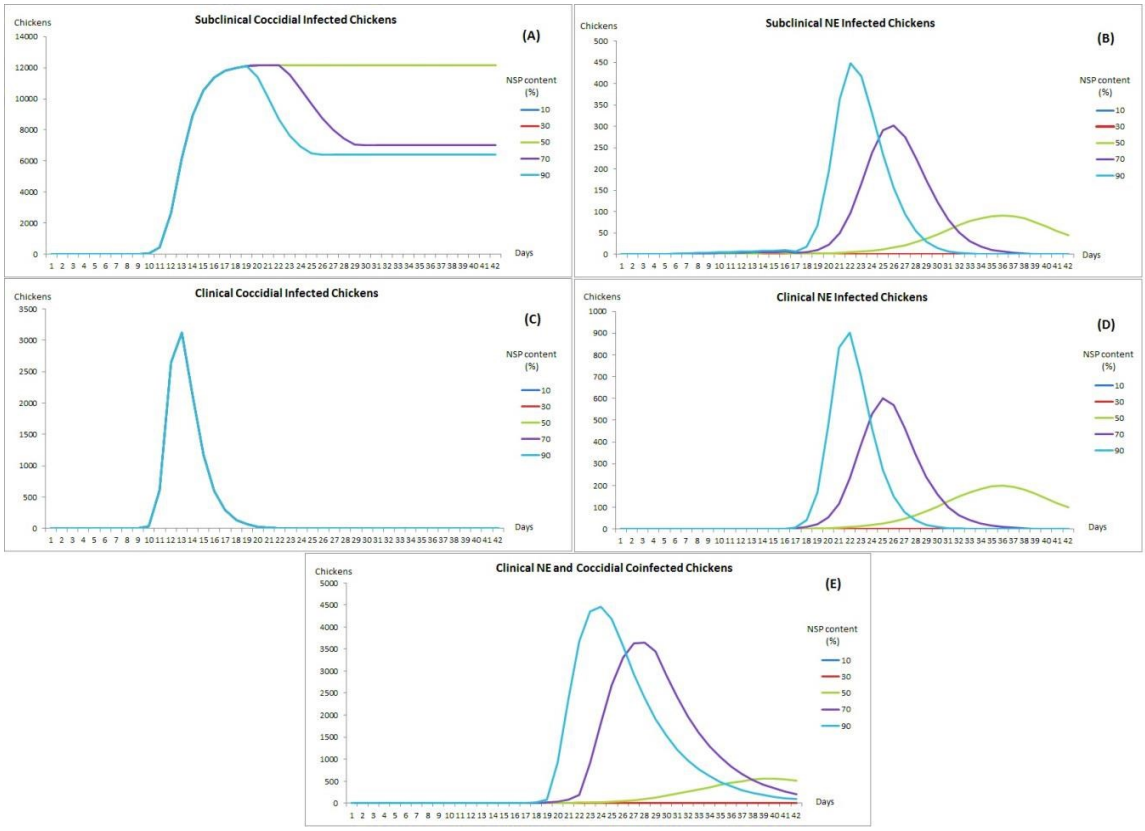

Figure 3.16 The scenario analysis of the feed NSP content (10 to 90%) and the incidence of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

Within a set of numbers ranging from 10% to 90% (the setting of feed NSP content was 60 %), the incidence of subclinical coccidiosis (A) after coinfection occurred, subclinical NE (B), clinical NE (D) and coinfection (E) increased when the feed NSP content increased, but there was no effect on subclinical coccidiosis (A) before coinfection occurred and clinical coccidiosis (C). After coinfection occurred, the dropping numbers of subclinical coccidial infected chickens were driven by coinfected chickens when the NSP content increased. (X axis: days, Y axis: the number of chickens)

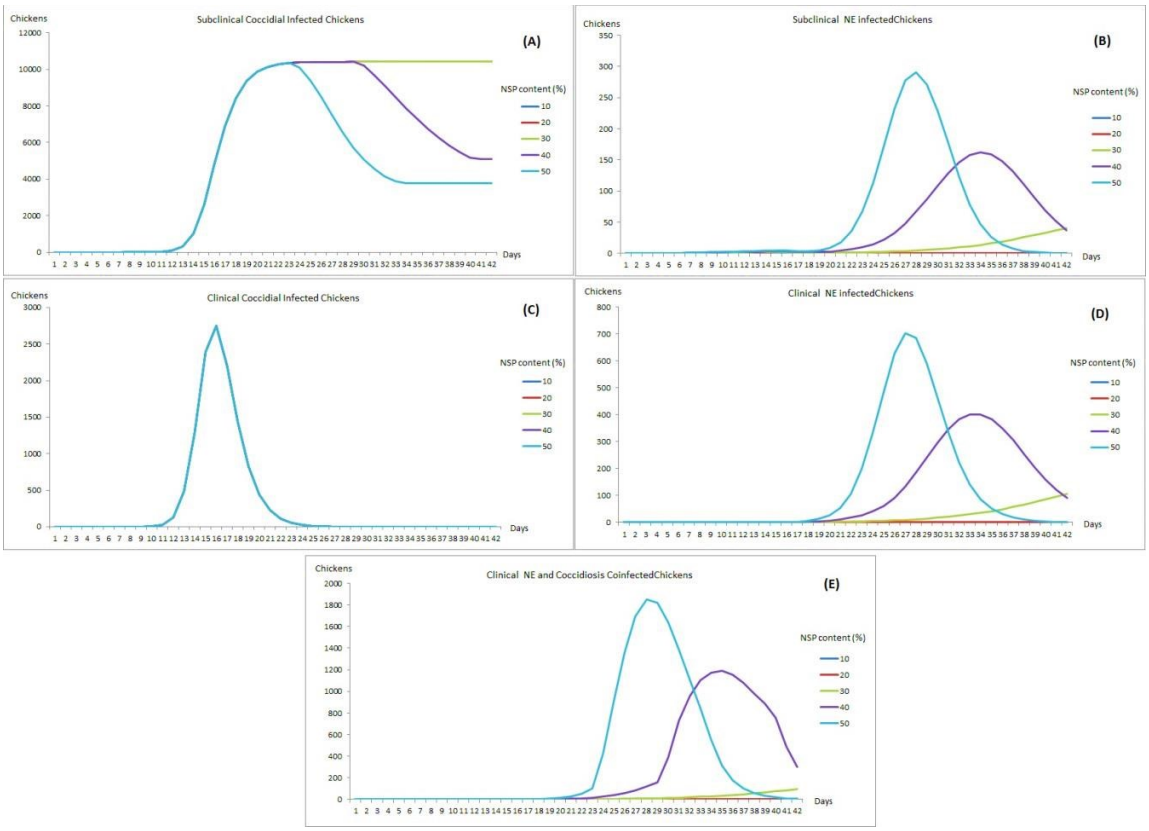

Figure 3.17 The scenario analysis of the feed NSP content (10 to 50%) and the incidence of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

Regarding the common application of NSP content ranges from 10 to 50%, a further analysis was conducted to see the variations. (X axis: days, Y axis: the number of chickens)

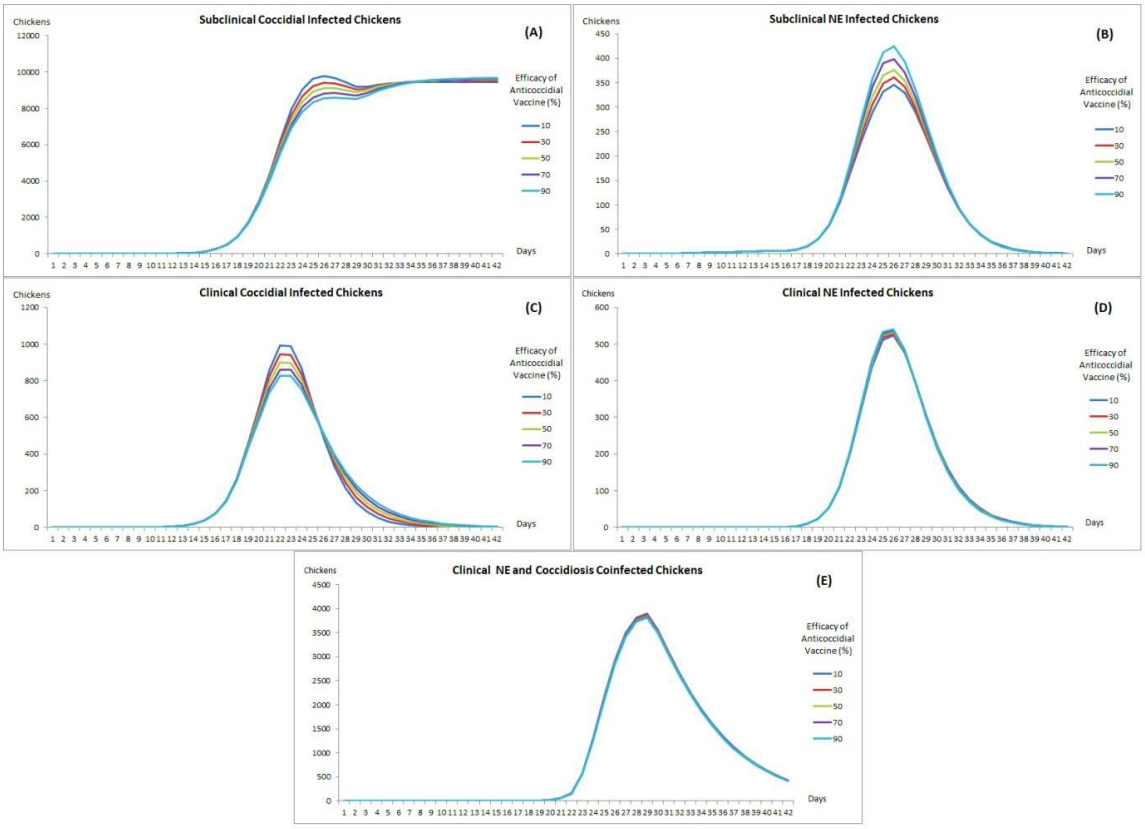

Figure 3.18 The scenario analysis of the efficacy of anticoccidial vaccine and the incidence of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

Within a set of numbers ranging from 10% to 90% (the setting of the efficacy of anticoccidial vaccine was 10%), the incidence of subclinical (A) and clinical coccidiosis (C) decreased when the efficacy of anticoccidial vaccine increased, but there was slightly inverse effects on subclinical NE (B), clinical NE (D) and coinfection (E). (X axis: days, Y axis: the number of chickens)

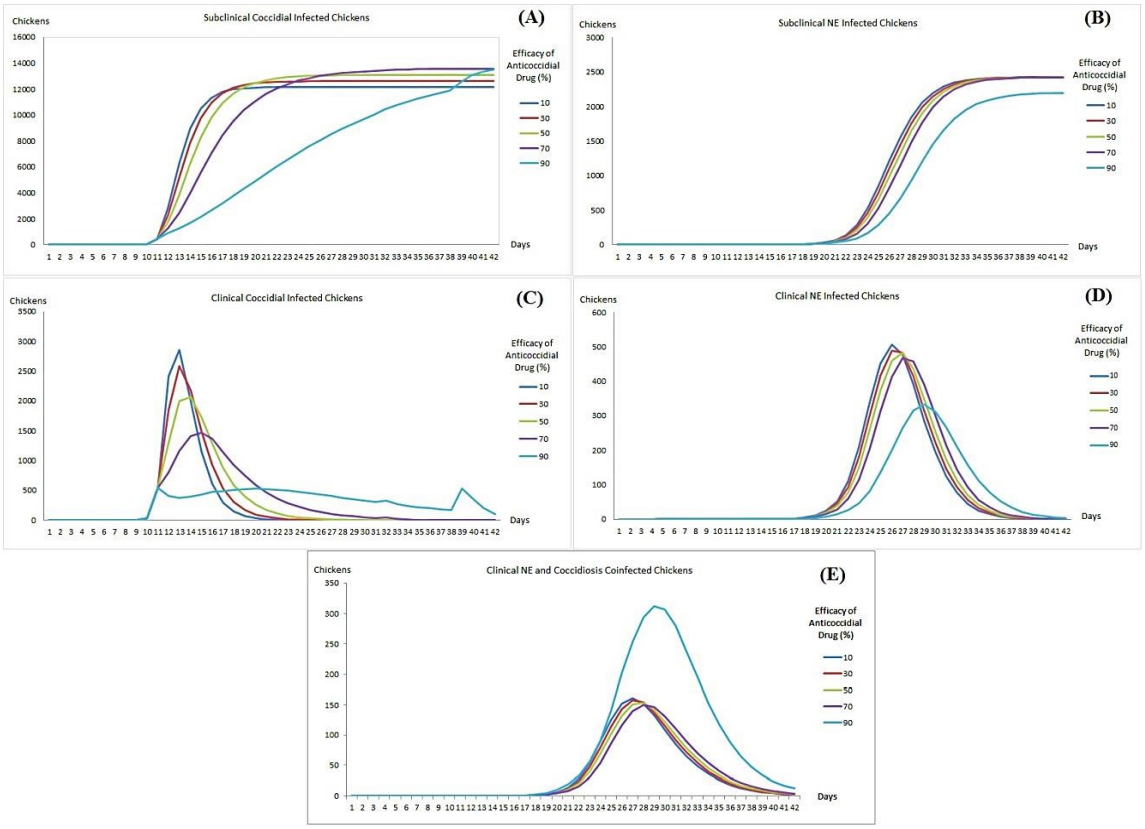

Figure 3.19 The scenario analysis of the efficacy of anticoccidial drug and the incidence of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

Within a set of numbers ranging from 10% to 90% (the setting of the efficacy of anticoccidial drug was 10%), the incidence of subclinical (A) and clinical coccidiosis (C) decreased when the efficacy of anticoccidial drug increased; besides, there was a comparatively little effect on subclinical NE (B), clinical NE (D) and coinfection (E), excluding the extreme condition of highest efficacy (90%) on coinfection. With highest efficacy, the coccidial infected population was dramatically decreased so that the majority of coinfected population was formed by merely the NE infected population which was also decreased under the effect of anticoccidial drug. (X axis: days, Y axis: the number of chickens)

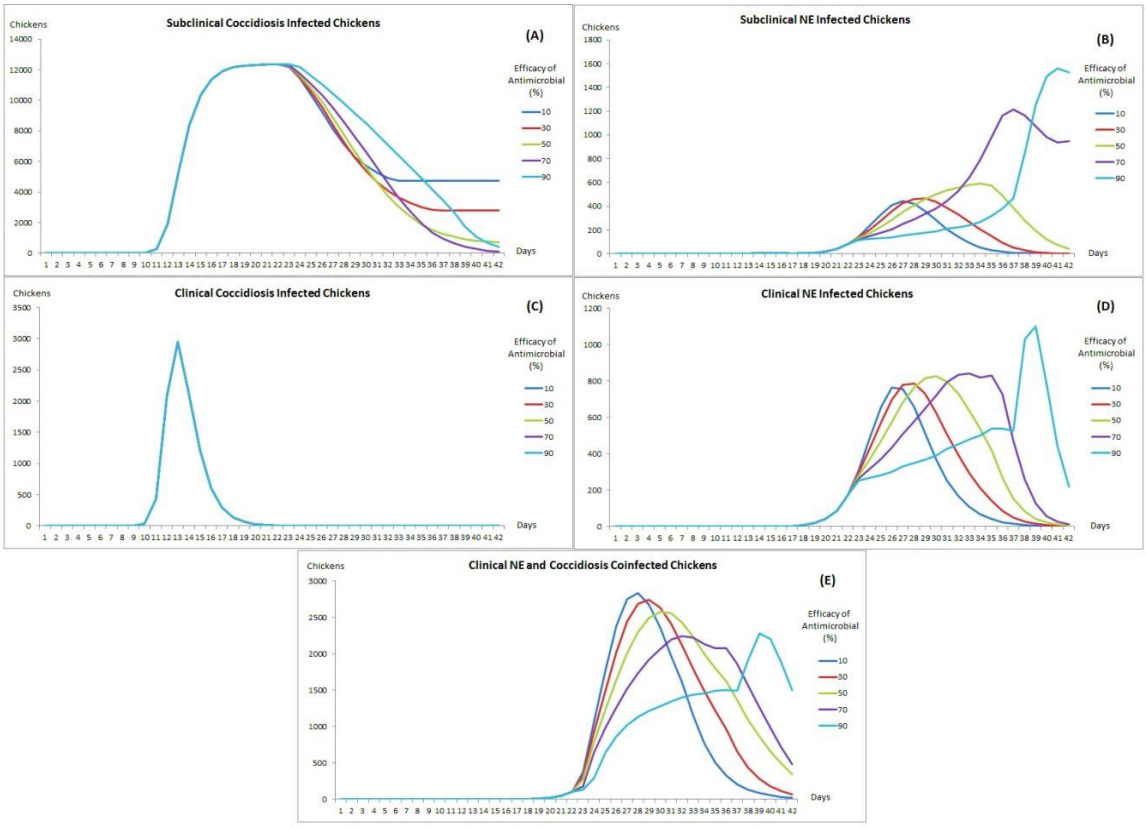

Figure 3.20 The scenario analysis of the efficacy of antimicrobial and the incidence of subclinical and clinical coccidiosis, subclinical and clinical NE and coinfection

Within a set of numbers ranging from 10% to 90% (the setting of the efficacy of antimicrobial was 10%), the incidence of subclinical coccidiosis (A) after coinfection occurred, subclinical NE (B), clinical NE (D) and coinfection (E) decreased when the efficacy of antimicrobial increased, but there was no effect on subclinical coccidiosis (A) before coinfection occurred and clinical coccidiosis (C). The dropping population of subclinical coccidiosis (A) after 21st day was driven by the increasing population of coinfection. The effect of antimicrobial on subclinical NE illustrated that the clinical NE infected chickens were turning into subclinical form under higher efficacy. The increasing numbers of chickens infected with coinfection showed a possible rebound corresponding to withdrawing (6 days before harvesting) the highest efficacy of antimicrobial. (X axis: days, Y axis: the number of chickens)

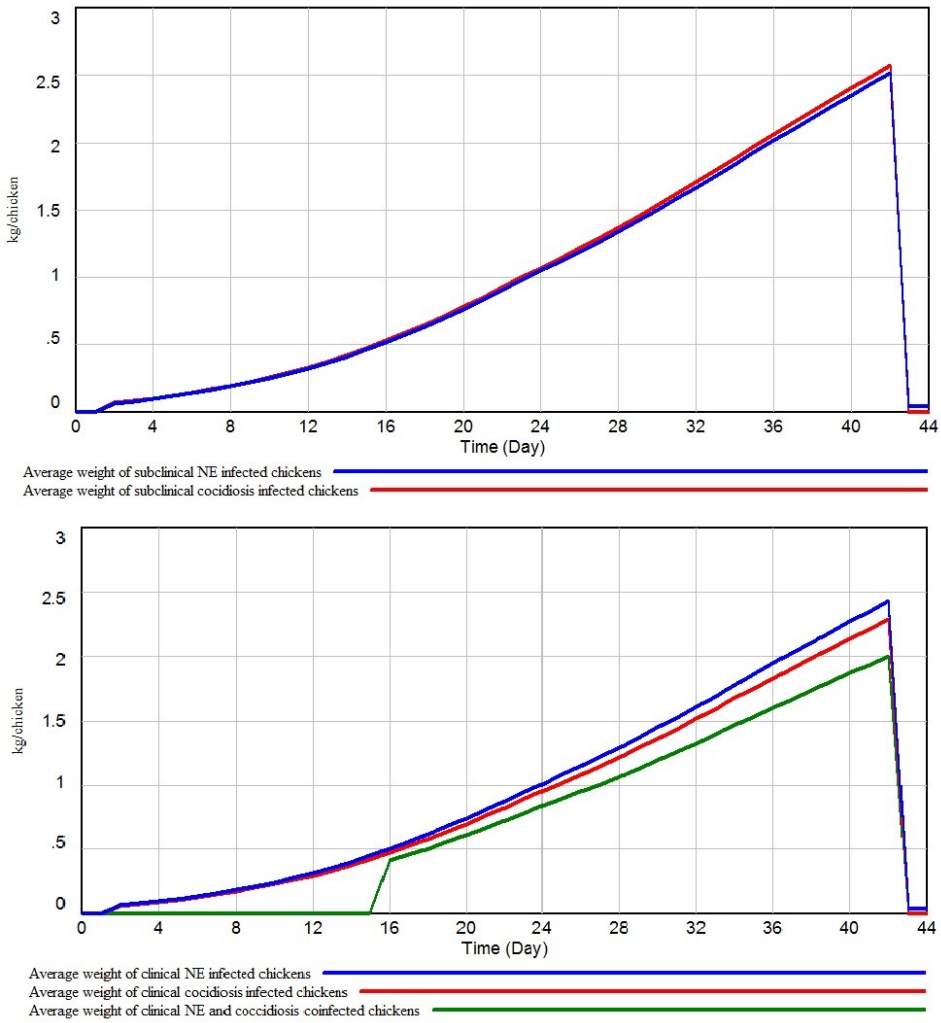

Figure 3.21 The patterns of average weights in chickens infected with subclinical form (A), and clinical form of NE, coccidiosis and coinfection (B) in one grow-out period

(A) As the populations of chickens infected with the subclinical NE and coccidiosis increased, patterns of gradually increasing average weights in these two groups were presented in one grow-out period. It showed the subclinical coccidial infected chickens were gaining more weight than subclinical NE infected chickens because of less weight loss in average.

(B) The average weights of chickens infected with clinical NE, clinical coccidiosis and coinfection accumulated differently representing variations in population sizes and individual weight loss.

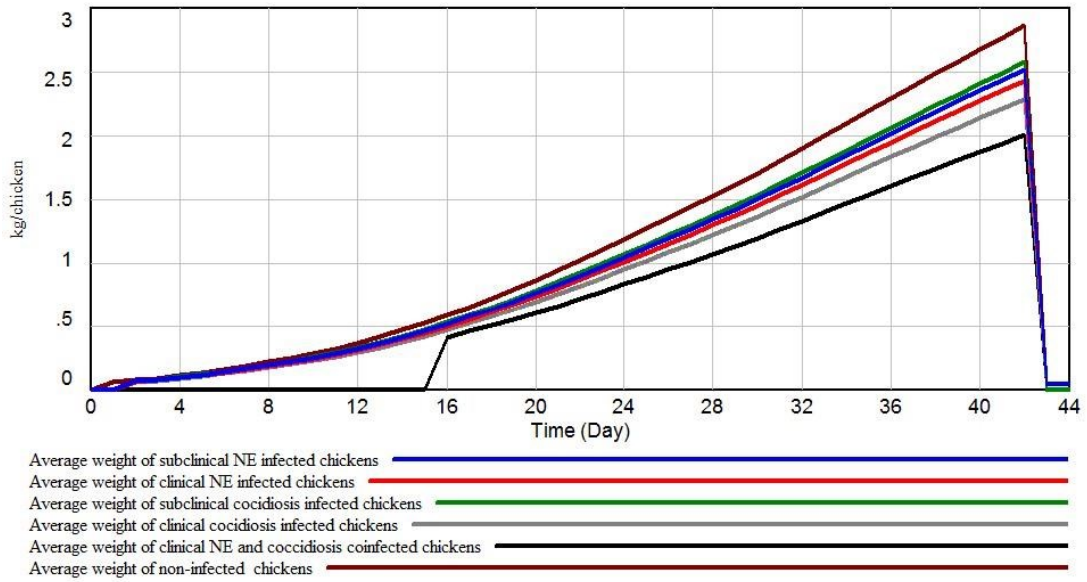

Figure 3.22 The patterns of average weights estimated in different populations in one grow-out period

The comparative patterns of average weights in chickens without infection and infected with the subclinical form, clinical form and concurrent infection of NE and coccidiosis were estimated in one grow-out period.

CHAPTER IV

CONCLUSIONS AND DISCUSSION

4.1 THE EPIDEMIOLOGY OF NE AND AVIAN COCCIDIOSIS

4.1.1 The overall development of disease epidemiology demonstrated a similarity of the system on a house level

The results of our stochastic models achieved the main purpose of this study which was to present the dynamic epidemiology of NE and how it would be affected by its predisposing factors in the system. The SD model displayed the patterns of exposure, transmission, progression in disease cycles of NE and avian coccidiosis in real time. The patterns were generated by the systematic ensemble of variables and matched the general observations on the occurrence and relationships of NE and coccidiosis as follows: (1) Chickens are most commonly affected with NE at 2 to 6 weeks old (Williams, 2005) and with coccidiosis at 3 to 6 weeks of age which seldom occurs at less than 11 days (McDougald and Long, 2003). It was reported that NE occurred at a median age of 26 days with an earliest onset at age of 10 days and a late one at age of 49 days (Hermans and Morgan, 2007); (2) Clinical coccidiosis predisposes birds to NE; also, severe coccidial lesions can occur alone before NE (Williams, 2003, 2005); (3) The occurrence of NE could be observed more than once on a farm in a year (Long, 1973). (4) In several experimental models, subclinical NE is likely to be detected in chickens at the age of 3 weeks (Kaldhusdal and Hofshagen, 1992; Wu et al., 2010); (5) Though there is no exact data of the prevalence of subclinical coccidiosis, it was estimated higher than the

prevalence of clinical form (Kadykalo et al., 2017); (6) The mortality of coccidiosis ranged from 6 to 87%, depends on chicken age, oocyst dosage and species (Levine, 1961); (7) The mortality of NE ranged from 0.5-1% under consecutive outbreaks on a farm; and the concurrent infection of coccidia could exacerbate the mortality up to 50% (Shane et al., 1985).

Furthermore, our SD model depicted the dynamic interactions of NE and avian coccidiosis under the hypothesis in which avian coccidiosis should affect NE from time to time. The stochastic arrangements of randomly choosing several exogenous variables, such as susceptibility, infectivity, starting days and case fatality, with means and standard deviations based on literature generated different values for each grow-out cycle. Thus, the disease model produced the dynamic variations of different populations which were presented the epidemiological diagrams of expectation. Meanwhile, by setting certain exogenous variables as explanatory variables, such as feed composition and efficacy of medication or vaccines, their values could be adjusted as interventions on predisposing factors so that the effects of interventions could be observed and evaluated. Besides, different average weights of each population, including infected and non-infected chickens, were estimated at the same time by the growth curve of broiler chickens and weight losses caused by NE, coccidiosis and coinfection. In one or multiple grow-out cycles, the submodel of average weights directly demonstrated the growth performance of the flock. In the future, this submodel could be expanded to an economic model so that cost and profit under influences of diseases could be evaluated in advance.

4.1.2 The findings of sensitivity and scenarios analyses revealed the probable leverage points of management practice

By applying the sensitivity analysis, several interesting findings were carried out regarding the contact rate, animal protein content, soy protein content and NSP content in feed which were considered as predisposing factors of NE. According to the sensitivity and scenario analyses of the contact rate, elevating the contact rate increased the incidence of clinical NE, coccidiosis and coinfection. This result was similar to the relationship of cluster density and contact frequency, the random movement and contact of individuals initially increases the frequency of contacts, especially high-density clusters, such as crowds at mass gatherings (Hu et al., 2013). Therefore, the effect of higher contact rate which came from the higher flock density or irregular crowding contributes as a risk factor to disease incidence in a chicken house. In respect to the analytic results of animal protein and NSP content in feed, they posed similar effects on the incidence of clinical NE and coinfection. As the content of both ingredients increased, the higher incidence was observed. The phenomenon that these two factors predisposed the occurrence of NE has been observed in field cases and experimental models (Brennan et al., 2003, 2001b; Kaldhusdal and Skjerve, 1996; Olkowski et al., 2006; Riddell and Kong, 1992). Inversely, elevating the content of soy protein in feed mitigated the incidence of NE which was mentioned in several experimental studies (Drew et al., 2004; Engberg et al., 2002; Furuse and Yokota, 1984; Williams et al., 2003). Mainly, the field or experimental research studies provided either qualitative or quantitative results; however, the current SD model offered the results of both types of data in a temporal view. Regarding the application of the anticoccidial vaccine and drugs, under higher efficacy, they provided greater protection for chickens from the infection of coccidiosis,

and some anticoccidial drugs provided protection against NE infection (Chapman, 2009, 1998; Williams et al., 2003, 1999). The use of anticoccidial vaccines is to some degree with controversy (Tabler et al., 2015). Some studies mentioned the observations of indirect protection given by anticoccidial vaccine to NE (Bangoura et al., 2014; Williams, 2005; Williams et al., 2003); however, a study observed a higher level of colonization of *Cl. perfringens* in the small intestine induced by concurrent infection of NE with a coccidial vaccine in comparison with uninfected controls, groups of *Cl. perfringens* infected alone or coccidial infected alone given by the anticoccidial vaccine (Pedersen et al., 2008). Also, the antimicrobial gave its protection to chickens and decreased the incidence of clinical NE. However, increasing the efficacy of medication decreased the incidence of clinical forms or coinfection. Especially under the highest efficacy of 90%, the populations of clinical NE infected and coinfected chickens were moved towards the subclinical infected chickens which the model presented as an unexpected phenomenon of using high dosage of antimicrobials. In addition, these analyzed results among critical factors provided the potential leverage points: (1) Decreasing the content of animal protein in feed which ranges from 9% to 1% could averagely decrease the peak population of clinical NE by 452chickens (669 to 217) and of coinfection by 2012 chickens (2326 to 314); (2) Minimizing the content of NSP in feed which ranges from 50% to 10%, decreased the peak population of clinical NE by 654 chickens (703 to 49) and of coinfection by 1749 chickens (1853 to 104); (3) Increasing the content of soy protein in feed which ranges from 10% to 50% averagely decreased the peak population of clinical NE by 434 chickens (529 to 116) and of coinfection by 1689 chickens (1780 to 91). With numerical changes, different levels of interventions on one predisposing factor

as well as their outcomes were obtained by the computing algorithm of the SD model; therefore, it demonstrated that different influences could be affected by different interventions on predisposing factors. Eventually, the influence of litter management is expected to be established in the SD model; however, the complete development requires further consideration and refinement.

4.2 THE GROWTH PERFORMANCE OF CHICKENS WITH/ WITHOUT INFECTION

The average weights in total or of different populations, such as infected chickens with or without clinical signs, were calculated continuously overtime. Based on the disease submodels, the growth performance of the single flock or multiple flocks was obtained. The average weights of different populations with infected and without infection estimated by the submodel were similar to what is expected in the real system. The weights of non-infected chickens averaged 6.3 lb. which reached the marketing weight, while the weights of infected chickens averaged 5.0 lb. (Table 3.3)

4.3 THE FUTURE APPLICATION

In this SD model developed for investigating the relationships and interactions of NE and its predisposing factors, we estimated the different populations of chickens infected with clinical and subclinical forms of NE and avian coccidiosis as well as the average weights of these populations. The SD model provided direct and persuasive outcomes in a more explicit way than models using statistical methodology. The interface of the model software presented the dynamic variations of the system over time and gave a better understanding of output. Moreover, based on the system thinking, it has a forecasting function generated from interactions that may or may not be observed in a

real system. Generally, there are two ways to develop the SD model after identifying the question of concerns. One is to collect data by conducting a survey or an interview, develop the model and analyze results to find leverage points. Another way is to develop the model with using literature values and then input data collected from the industry or the field. With continuous refinement of the model, constructive suggestions can be provided. Our current model belonged to the second type of model establishment. However, lacking empirical data collected in the field prevented the stochastic model from providing expected results for specific farms or expanding its application to include several farms as a complex with further comparisons among them.

In the current study, our goals of reflecting the epidemiology of NE and avian coccidiosis as well as finding risk factors in management practices which could decrease the incidence of diseases were achieved. Further studies is proposed as follows: Firstly, epidemiological data will be collected from the broiler industry by conducting a survey which covers information regarding the feed program, medication programs, management practices and laboratory diagnosis at the outbreaks of NE in farms or a complex. Secondly, after utilizing the data with continuous refinement of model, these parameters will be replaced with stochastic variables and processing the relevant analyses to seek the leverage points of decreasing the occurrence of NE in specific farms or a complex. Thirdly, an economic submodel will be developed for the profit analyses by applying the market prices to the submodel of average weights under the influences of NE and avian coccidiosis. The entire SD model is expected to look for the balances of inventory and profit while adjusting the policies of management practices as well as the programs of medication and feed.

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APPENDIX A

THE LISTS OF EXOGENOUS VARIABLES, ENDOGENOUS VARIABLES AND ABBREVIATIONS OF TERMS

App.1 The list of exogenous variables

No.	Variable	Value	Unit
1	CHICKENS PER DAY	1.00	1/day
	A variable for converting units		
2	COCCIDIAL INITIAL INFECTED	1.00	chickens
	The first chicken infected with avian coccidia		
3	COCCIDIOSIS INFECTIOUS PERIOD	7.00	Days
	The duration of chickens infected with clinical coccidiosis became subclinical form with infectivity/ The prepatent period. (Levine, 1961)		
4	COCCIDIOSIS MORBIDITY TRIGGER	0.02	Dmnl
	The morbidity of clinical coccidiosis infection that triggers the anticoccidial drug administration		
5	CONTACT RATE	10.00	1/day
	The rate of increase of new infective cases occurs in proportion to the product of the number of susceptible and the number of infected individual (Rhodes, 2008		
6	DAY OLD CHICK BODY WEIGHT	40.00	Gram
	The average body weight of day-old chick. (Ross and Cobb broiler management guidelines)		
7	DELIVERY PER DAY	1	1/day
	Chickens delivered everyday		
8	DELIVERY SIZE	20000	Chickens
	The size of chickens delivered per grow-out cycle		
9	DOWN TIME	14.00	Days
	Downtime: The period between flocks, starting with a barn or flock area being emptied of birds and ending with the placement of new birds/ Ideally, this should be at least 14 days to allow adequate time for pathogen reduction. (National Avian On-Farm Biosecurity Standard, 2013.)		
10	EFFICACY OF ANTICOCCIDIAL DRUGS	0.10	Dmnl
	It gives the effect of the application of antimicrobials which depends on types of antimicrobials and their dosage/ The range was assumed as greater than 0 to less than 1.		
11	EFFICACY OF ANTIMICROBIAL	0.10	Dmnl
	It gives the effect of the application of anticoccidial drugs and depends on type of anticoccidial drugs and their dosage/ The range was assumed as greater than 0 to less than 1.		
12	EFFICACY OF VACCINE ADMISTRATION	0.10	Dmnl
	It gives the effect of the application of anticoccidial drugs which depends on type of anticoccidial vaccines and administrations/ The range was assumed as greater than 0 to less than 1		
13	FEED ANIMAL PROTEIN CONTENT	0.03	Dmnl
	The percentage of animal protein content in feed (Pedersen, 2003)		
14	FEED NSP CONTENT	0.60	Dmnl

	The percentage of NSP content in feed. This value and the value of feed corn content were added as 1 (Branton, 1987)		
15	FEED SOY PROTEIN CONTENT	0.24	Dmnl
	The percentage of soybean protein content in feed (Pedersen, 2003)		
16	FINAL TIME	42.00	Days
	The end day of simulation		
17	GO PERIOD	42.00	Days
	The end day of grow-out period (USDA APHIS FAD PReP Industry Manuals, 2013)		
18	KG PER GRAM= 1/1000	1/1000	kg/gram
	To convert the weight to Kilogram		
19	LITTER REMOVAL FREQUENCY	1.00	Cycle
	This is the frequency of litter removal or every certain number of cycles		
20	MEAN COCCIDIAL CASE FATALITY	0.19	Dmnl
	The number of deaths in chickens infected with coccidiosis (Mayhew, 1933; Waletzky and Hughes, 1949; Horton-Smith, 1949; Amer, 2010; Bangoura, 2014)		
21	MEAN COCCIDIOSIS INFECTIVITY	0.41	Dmnl
	The number of chickens infected with coccidiosis (Peek, 2003; Györke, 2003; Haug, 2008; Ogedengbe, 2011; Gharekhani, 2014)		
22	MEAN COCCIDIOSIS START DAY	8.00	Days
	The day of first chicken infected with coccidiosis (Tyzzer 1929; Levine 1942; Brackett, 1952; Gordeuk, 1951; Gardiner, 1995)		
23	MEAN NE AND COCCIDIAL CASE FATALITY	0.51	Dmnl
	The number of deaths in chickens infected with clinical NE and coccidiosis (Al-Sheikhly, 1980; Shane, 1985; Baba, 1997; Williams, 2003; Park, 2008)		
24	MEAN NE CASE FATALITY	0.06	Dmnl
	The number of deaths in chickens infected with clinical NE (Nairn, 1967; Long, 1976; Shane, 1985; Kaldhusdal, 1992; Kaldhusdal, 1999)		
25	MEAN NE INFECTIVITY	0.46	Dmnl
	The number of chickens infected with clinical NE (Kaldhusdal, 1996; Brennan 2001; Lovland 2003; Dahiya, 2006; Cooper, 2010)		
26	MEAN NE START DAY	15.00	Days
	The day of first chicken infected with NE (Parish, 1961; Shane, 1985; Riddell, 1992; Kaldhusda, 1999; Craven, 2000)		
27	MEAN SUBCLINICAL COCCIDIOSIS FRACTION*	0.26	Dmnl
	The number of chickens infected with subclinical coccidiosis (Reza Razmi, 2000; Peek, 2003; Kadykalo, 2017)		
28	MEAN SUBCLINICAL NE FRACTION	0.25	Dmnl
	The number of chickens infected with subclinical NE (Kaldhusdal, 1992; Lovland, 2003; Fernando, 2011)		
29	MEAN SUSCEPTIBILITY	0.73	Dmnl
	The percentage of chickens susceptible to the disease (Mayhew, 1933; Amer, 2010; Grenier, 2016)		

30	NE INFECTIOUS PERIOD	7.00	Days
	The duration of chickens infected with clinical NE became subclinical form with infectivity (Helmboldt, 1971)		
31	NE INITIAL INFECTED	1.00	Chickens
	The initial NE infected chickens		
32	NE MORBIDITY TRIGGER	0.02	Dmnl
	The morbidity of clinical NE infection that triggers the antimicrobial administration		
33	PER CYCLE	1.00	1/cycle
	To uniform the units		
34	PERIOD OF ANTICOCCIDIAL DRUGS ADMINISTRATION	21.00	Days
	The duration of drug administration (Kant, 2013)		
35	PERIOD OF ANTICOCCIDIAL DRUGS WITHDRAWL	5.00	Days
	The duration of withdrawing the drug administration before harvesting (Duquette, 2005)		
36	PERIOD OF ANTIMICROBIAL ADMINISTRATION	5.00	Days
	The duration of drug administration (Lanckriet, 2010)		
37	PERIOD OF ANTIMICROBIAL WITHDRAWAL	6.00	Days
	The duration of withdrawing the drug administration before harvesting (Shojadoost , 2013)		
38	PROPORTION THRESHOLD OF COCCIDIOSIS	0.10	Dmnl
	The proportion thresholds of coccidiosis was assumed to be the initial proportion of infected chickens in the population that must exceed a certain number, which depends upon the coccidial infectivity, to cause the occurrence of coinfection (Jeffers, 1974)		
39	PROPORTION THRESHOLD OF NE	0.05	Dmnl
	The proportion thresholds of NE was assumed to be the initial proportion of infected chickens in the population that must exceed a certain number, which depends upon the NE infectivity, to cause the occurrence of coinfection (Shane, 1985)		
40	SEED	0.00	Dmnl
	To generate alternative noise streams in different simulations		
41	STDDEV COCCIDIAL CASE FATALITY	0.09	Dmnl
	The standard deviation of average deaths in chickens infected with clinical coccidiosis (Mayhew, 1933; Waletzky and Hughes, 1949; Horton-Smith, 1949; Amer, 2010; Bangoura, 2014)		
42	STDDEV COCCIDIOSIS INFECTIVITY	0.19	Dmnl
	The standard deviation of average number in chickens infected with clinical coccidiosis (Peek, 2003; Györke, 2003; Haug, 2008; Ogedengbe, 2011; Gharekhani, 2014)		
43	STDDEV COCCIDIOSIS START DAY	0.04	Dmnl

	The standard deviation of average days of first chicken infected with coccidiosis (Tyzzer 1929; Levine 1942; Brackett, 1952; Gordeuk, 1951; Gardiner, 1995)		
44	STDDEV NE AND COCCIDIAL CASE FATALITY	0.24	Dmnl
	The standard deviation of average deaths in chickens coinfected with clinical NE and coccidiosis (Sheikhly, 1980; Shane, 1985; Baba, 1997; Williams, 2003; Park, 2008)		
45	STDDEV NE CASE FATALITY	0.04	Dmnl
	The standard deviation of average deaths in chickens infected with clinical NE (Nairn, 1967; Long, 1976; Shane, 1985; Kaldhusdal, 1992; Kaldhusdal, 1999)		
46	STDDEV NE INFECTIVITY	0.20	Dmnl
	The standard deviation of average number in chickens infected with clinical NE (Kaldhusdal, 1996; Brennan 2001; Lovland 2003; Dahiya, 2006; Cooper, 2010)		
47	STDDEV NE START DAY	0.01	Dmnl
	The standard deviation of average days of first chicken infected with NE (Parish, 1961; Shane, 1985; Riddell, 1992; Kaldhusda, 1999; Craven, 2000)		
48	STDDEV SUBCLINICAL COCCIDIOSIS FRACTION	0.10	Dmnl
	The standard deviation of average number in chickens infected with clinical coccidiosis (Reza Razmi, 2000; Peek, 2003; Kadykalo, 2017)		
49	STDDEV SUBCLINICAL NE FRACTION	0.05	Dmnl
	The standard deviation of average number in chickens infected with subclinical NE (Kaldhusdal, 1992; Lovland, 2003; Fernando, 2011)		
50	STDDEV SUSCEPTIBILITY	0.40	Dmnl
	The standard deviation of average number in chickens susceptible to *Eimeria* spp. (Mayhew, 1933; Amer, 2010; Grenier, 2016)		
51	THE DAY OF VACCINE ADMINISTRATION	5.00	Days
	The starting day of vaccine administration (Williams, 1999)		
52	TIME STEP	0.06	Days
	The time step for the simulation		
53	WEIGHT LOSS OF CLINICAL COCCIDIOSIS INFECTED CHICKENS	0.20	Dmnl
	The percentage of total weight loss due to the clinical coccidiosis (Alnassan, 2014)		
54	WEIGHT LOSS OF CLINICAL NE AND COCCIDIOSIS COINFECTED CHICKENS	0.30	Dmnl
	The percentage of total weight loss due to the clinical NE and coccidiosis coinfection (Alnassan, 2014)		
55	WEIGHT LOSS OF CLINICAL NE INFECTED CHICKENS	0.15	Dmnl
	The percentage of total weight loss due to the clinical NE (Alnassan, 2014)		
56	WEIGHT LOSS OF SUBCLINICAL COCCIDIOSIS INFECTED CHICKENS	0.10	Dmnl

	The percentage of total weight loss due to the subclinical coccidiosis (Kandeel, 2011)		
57	WEIGHT LOSS OF SUBCLINICAL NE INFECTED CHICKENS	0.12	Dmnl
	The percentage of total weight loss due to the subclinical NE (Qing, 2017)		

Exogenous variables: factors in a causal model or causal system under study whose value is independent from the states of other variables in the system; a factor whose value is determined by factors or variables outside the system.

App.2 The list of endogenous variables

No.	Variable	Unit
1	Age of chicken	Days
	= IF THEN ELSE (Cycle day<=GO PERIOD, Cycle day, 0)	
2	Anticoccidial Drugs Administration	1
	= INTEG (starting administration of anticoccidial drugs-ending administration of anticoccidial drugs, 0)	
3	Antimicrobial Administration	1
	= INTEG (starting antimicrobial administration-ending antimicrobial administration, 0)	
4	Application of anticoccidial drugs	Dmnl
	= IF THEN ELSE ((GO PERIOD-Cycle day>PERIOD OF ANTICOCCIDIAL DRUGS WITHDRAWL), Need for anticoccidial drugs administration*0.9, 0)	
5	Application of antimicrobial	1
	= IF THEN ELSE ((GO PERIOD-Cycle day)>PERIOD OF ANTIMICROBIAL WITHDRAWAL, Need for antimicrobial administration* 0.9, 0)	
6	Average weight of clinical coccidiosis infected chickens	Kg/chicken
	= XIDZ (Weight of clinical coccidiosis infected chickens, Clinical Coccidial Infected Chickens, 0)	
7	Average weight of clinical NE and coccidiosis coinfected chickens	Kg/chicken
	= XIDZ (Weight of clinical NE and coccidiosis coinfected chickens, Clinical NE and Coccidiosis Coinfected Chickens, 0)	
8	Average weight of clinical NE infected chickens	Kg/chicken
	= XIDZ (Weight of clinical NE infected chickens, Clinical NE Infected Chickens, 0)	
9	Average weight of grow-out chickens	Kg/chicken
	= XIDZ (Weight of other grow-out chicken, (Grow Out Chickens-Infected Chickens-Dead Chickens), 0)	
10	Average weight of subclinical coccidiosis infected chickens	Kg/chicken
	= XIDZ (Weight of subclinical coccidiosis infected chickens, Subclinical Coccidial Infected Chickens, 0)	
11	Average weight of subclinical NE infected chickens	Kg/chicken
	= XIDZ (Weight of subclinical NE infected chickens, Subclinical NE Infected Chickens, 0)	
12	Average Weight per day	Gram/chicken
	= WITH LOOKUP (Age of chicken)	
	http://www.cobb-vantress.com//docs/default source/cobb500guides/Cobb500_ Broiler_Performance_And_Nutrition_Supplement.pdf:cobb500 broiler as hatched. 2015	
13	chickens become susceptible to Clostridium Perfringens	Chickens/day
	= Initial susceptible population to Clostridium perfringens*DELIVERY PER DAY	

14	chickens become susceptible to Eimeria spp.	Chickens/day
	= DELIVERY PER DAY*Initial susceptible population to Eimeria spp.*PULSE TRAIN (1, 1, Cycle period, FINAL TIME)	
15	chickens infected with clinical coccidiosis coinfected with NE	Chickens/day
	= IF THEN ELSE(Effective coccidial fatality + Fraction of coccidial infected chickens coinfected with NE>=1, 1-Effective coccidial fatality, Fraction of coccidial infected chickens coinfected with NE)*Clinical Coccidial Infected Chickens*Effect of anticoccidial drugs on coccidiosis*CHICKENS PER DAY	
16	chickens infected with clinical NE coinfected with coccidiosis	Chickens/day
	= IF THEN ELSE(Effective NE fatality + Fraction of NE infected chickens coinfected with coccidiosis>=1,1-Effective NE fatality, Fraction of NE infected chickens coinfected with coccidiosis)*Clinical NE Infected Chickens*Effect of antimicrobial on NE*CHICKENS PER DAY	
17	Clinical NE Dead Chickens	Chickens
	= INTEG (dying clinical NE infected chickens-removing clinical NE dead chickens, 0)	
18	Clinical Coccidial Infected Chickens	Chickens
	= INTEG (developing of clinical coccidial infected chickens-chickens infected with clinical coccidiosis coinfected with NE-dying clinical coccidial infected chickens-harvesting clinical coccidial chickens-transforming of clinical coccidial to subclinical coccidial infected chickens, 0)	
19	coccidiosis chickens coinfected with NE	Chickens/day
	= chickens infected with clinical coccidiosis coinfected with NE + chickens infected with subclinical coccidiosis coinfected with NE	
20	Clinical Coccidiosis Dead Chickens	Chickens
	= INTEG (dying clinical coccidial infected chickens-removing clinical coccidial dead chickens, 0)	
21	Clinical NE and Coccidiosis Coinfected Chickens	Chickens
	= Clinical NE and Coccidiosis Coinfected Chickens= INTEG (coccidiosis chickens coinfected with NE+NE infected chickens coinfected with coccidiosis-dying clinical NE and coccidial coinfected chickens-harvesting clinical NE and coccidiosis coinfected chickens, 0)	
22	Clinical NE and Coccidiosis Coinfected Dead Chickens	Chickens
	= Clinical NE and Coccidiosis Coinfected Dead Chickens= INTEG (dying clinical NE and coccidial coinfected chickens-removing clinical NE and coccidial coinfected dead chickens, 0)	
23	Clinical NE Infected Chickens	Chickens
	= INTEG (developing of clinical NE infected chickens-chickens infected with clinical NE coinfected with coccidiosis-dying clinical NE infected chickens-harvesting clinical NE infected chickens-transforming of clinical NE to subclinical NE infected chickens, 0)	
24	NE infected chickens coinfected with coccidiosis	Chickens/ day
	chickens infected with clinical NE coinfected with coccidiosis + chickens infected with subclinical NE coinfected with coccidiosis	
25	Coccidiosis effective contacts	Chickens/day

	= Coccidiosis susceptible contacts*Fraction of coccidial infected chickens* Effect of anticoccidial drugs on coccidiosis	
26	Coccidiosis infectivity	Dmnl
	= Coccidiosis infectivity for cycle	
27	Coccidiosis infectivity for cycle	Dmnl
	= SAMPLE IF TRUE (Cycle day=1, Random coccidiosis infectivity, 1e-05)	
28	Coccidiosis start day	Days
	= Coccidiosis start day for cycle	
29	Coccidiosis start day for cycle	Days
	=SAMPLE IF TRUE (Cycle day=1, Random coccidiosis start day, 1e-05)	
30	Coccidiosis susceptible contacts	Chickens/day
	= CONTACT RATE*Susceptible Chickens to Eimeria spp.*(1-Fraction chickens with coccidial protective immunity)	
31	Cycle day	Days
	= MODULO (Time, GO PERIOD+DOWN TIME)	
32	Cycle period	Days
	= DOWN TIME+GO PERIOD	
33	Dead Chickens	Chickens
	= Clinical NE Dead Chickens +Clinical Coccidiosis Dead Chickens +Clinical NE and Coccidiosis Coinfected Dead Chickens	
34	deaths due to coccidiosis	Chickens/day
	= dying clinical coccidial infected chickens +dying clinical NE and coccidial coinfected chickens	
35	deaths due to NE	Chickens/day
	= dying clinical NE infected chickens +dying clinical NE and coccidial coinfected chickens	
36	delivering chickens	Chickens/day
	= DELIVERY PER DAY*DELIVERY SIZE*PULSE TRAIN (0, 1, Cycle period, FINAL TIME)	
37	developing of clinical coccidial infected chickens	Chickens/day
	= incubated Eimeria spp. Infected Chickens*(1-Fraction of subclinical coccidial infected chickens) *Effect of anticoccidial drugs on coccidiosis* CHICKENS PER DAY	
38	developing of clinical NE infected chickens	Chickens/day
	= Incubated Pathogenic Clostridium Perfringens Infected Chickens*(1-Fraction of subclinical NE infected chickens) *Go end day*Effect of antimicrobial on NE*CHICKENS PER DAY	
39	developing of subclinical coccidial infected chickens	Chickens/day
	= incubated Eimeria spp. Infected Chickens*Fraction of subclinical coccidial infected chickens*Effect of anticoccidial drugs on coccidiosis*CHICKENS PER DAY	
40	developing of subclinical NE infected chickens	Chickens/day
	= Incubated Pathogenic Clostridium Perfringens Infected Chickens*Fraction of subclinical NE infected chickens*Effect of antimicrobial on NE	

41	dying clinical coccidial infected chickens	Chickens/day
	= Clinical Coccidial Infected Chickens*Effective coccidial fatality *Effect of anticoccidial drugs on coccidiosis*CHICKENS PER DAY	
42	dying clinical NE and coccidial coinfected chickens	Chickens/day
	= Clinical NE and Coccidiosis Coinfected Chickens*Effective NE and coccidial fatality*CHICKENS PER DAY	
43	dying clinical NE infected chickens	Chickens/day
	= Clinical NE Infected Chickens*Effective NE fatality *Effect of antimicrobial on NE*CHICKENS PER DAY	
44	Effect of anticoccidial drugs on coccidiosis	Dmnl
	= 1-(Application of anticoccidial drugs/0.9*EFFICACY OFANTICOCCIDIAL DRUGS)	
45	Effect of antimicrobial on NE	Dmnl
	= 1-(Application of antimicrobial/0.9*EFFICACY OF ANTIMICROBIAL)	
46	Effect of coccidiosis	Dmnl
	= IF THEN ELSE (Proportion coccidial infected chickens>PROPORTION THRESHOLD OF COCCIDIOSIS, Proportion coccidial infected chickens, PROPORTION THRESHOLD OF COCCIDIOSIS)	
	This is the effect of coccidia on *Cl. perfringens*. If the prevalence of coccidial infected birds is greater than the threshold, then there is an increase in the infectivity of *Cl. perfringens*.	
47	Effect of feed composition	Dmnl
	= Ratio of animal/soybean protein in feed + Ratio of NSP/corn in feed	
48	Effect of NE	Dmnl
	IF THEN ELSE(Proportion NE infected chickens>PROPORTION THRESHOLD OF NE, Proportion NE infected chickens, 0)	
	This is the effect of *Cl. perfringens* on coccidia. If the prevalence of infected *Cl. perfringens* birds is greater than the threshold, then there is an increase in the infectivity of coccidial.	
49	Effective coccidial fatality	Dmnl
	= Effective coccidial fatality for cycle	
50	Effective coccidial fatality for cycle	Dmnl
	= SAMPLE IF TRUE (Cycle day=1, Random effective coccidial fatality, 1e-05)	
51	Effective NE and coccidial fatality	Dmnl
	= Effective NE and coccidial fatality for cycle	
52	Effective NE and coccidial fatality for cycle	
	= SAMPLE IF TRUE (Cycle day=1, Random effective NE and coccidial fatality, 1e-05)	
53	Effective NE fatality	Dmnl
	= Effective NE fatality for cycle	
54	Effective NE fatality for cycle	
	= SAMPLE IF TRUE (Cycle day=1, Random effective NE fatality, 1e-05)	
55	ending administration of anticoccidial drugs	1/day

	= DELAY FIXED (starting administration of anticoccidial drugs, PERIOD OF ANTICOCCIDIAL DRUGS ADMINISTRATION -1, starting administration of anticoccidial drugs)	
56	ending antimicrobial administration	1/day
	= DELAY FIXED (starting antimicrobial administration, PERIOD OF ANTIMICROBIAL ADMINISTRATION-1, 0)	
57	Fraction chickens with coccidial protective immunity	Dmnl
	= IF THEN ELSE (Cycle day>=THE DAY OF VACCINE ADMINISTRATION, Proportion chickens with coccidial protective immunity* Go end day, 0)	
58	Fraction of coccidial infected chickens	Dmnl
	= IF THEN ELSE (Cycle day<Coccidiosis start day, ZIDZ (COCCIDIAL INITIAL INFECTED, Grow Out Chickens), IF THEN ELSE (Cycle day>=Coccidiosis start day, ZIDZ (Incubated Eimeria spp. Infected Chickens +Clinical Coccidial Infected Chickens + Subclinical Coccidial Infected Chickens, Grow Out Chickens) *Go end day, 0))	
59	Fraction of NE infected chickens coinfected with coccidiosis	Dmnl
	= IF THEN ELSE(Cycle day>=NE start day, Effect of coccidiosis*Go end day, 0)	
60	Fraction of coccidial infected chickens coinfected with NE	Dmnl
	= IF THEN ELSE(Cycle day>=Coccidiosis start day, Effect of NE*Go end day, 0)	
61	Fraction of NE infected chickens	Dmnl
	= IF THEN ELSE (Cycle day<NE start day, ZIDZ (NE INITIAL INFECTED, Grow Out Chickens) *Go end day, IF THEN ELSE(Cycle day>=NE start day, ZIDZ(Incubated Pathogenic Clostridium Perfringens Infected Chickens +Subclinical NE Infected Chickens +Clinical NE Infected Chickens, Grow Out Chickens)*Go end day, 0))	
	Proportion of birds in grow-out population that are infected with *Cl. Perfringens*.	
62	Fraction of subclinical coccidial infected chickens	Dmnl
	= IF THEN ELSE(Cycle day>=Coccidiosis start day, Subclinical coccidiosis fraction*Go end day, 0)	
63	Fraction of subclinical NE infected chickens	Dmnl
	= IF THEN ELSE(Cycle day>=NE start day, Subclinical NE fraction*Go end day, 0)	
64	Go end day	Dmnl
	= IF THEN ELSE (Cycle day>=GO PERIOD, 0, 1)	
65	Grow Out Chickens	Chickens
	= INTEG (delivering chickens-deaths due to coccidiosis-deaths due to NE-harvesting chickens, 0)	
66	Harvested Chickens	Chickens
	= INTEG (harvesting chickens-transporting chickens to processing plants, 0)	
67	harvesting chickens	Chickens

	= (Grow Out Chickens/TIME STEP) *PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
68	harvesting clinical coccidial chickens	Chickens/day
	= (Clinical Coccidial Infected Chickens/TIME STEP) *PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
69	harvesting clinical NE and coccidiosis coinfected chickens	Chickens/day
	= Clinical NE and Coccidiosis Coinfected Chickens/TIME STEP*PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
70	harvesting clinical NE infected chickens	Chickens/day
	= Clinical NE Infected Chickens/TIME STEP*PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
71	harvesting incubated Eimeria spp. infected Chickens	Chickens/day
	= Incubated Eimeria spp. Infected Chickens/TIME STEP*PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
72	harvesting incubated pathogenic Clostridium perfringens infected chickens	Chickens/day
	= Incubated Pathogenic Clostridium Perfringens Infected Chickens/TIME STEP*PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
73	harvesting NE susceptible chickens	Chickens/day
	= (Susceptible Chickens to Pathogenic Clostridium Perfringens/TIME STEP) *PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
74	harvesting subclinical coccidial chickens	Chickens/day
	= (Subclinical Coccidial Infected Chickens/TIME STEP) *PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
75	harvesting subclinical NE chickens	Chickens/day
	= (Subclinical NE Infected Chickens/TIME STEP) *PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
76	harvesting susceptible chickens to coccidiosis	Chickens/day
	= Susceptible Chickens to Eimeria spp./TIME STEP*PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
77	Incubated Eimeria spp. Infected Chickens	Chickens
	= INTEG (infecting of Eimeria spp. susceptible chickens-developing of clinical coccidial infected chickens -developing of subclinical coccidial infected chickens-harvesting incubated Eimeria spp. infected Chickens -incubated Eimeria spp. chickens coinfected with Clostridium perfringens, 0)	
78	incubated pathogenic Clostridium perfringens chickens coinfected with coccidiosis	Chickens/day
	= Incubated Pathogenic Clostridium Perfringens Infected Chickens*Fraction of incubated Clostridium perfringens chickens coinfected with coccidia*Effect of antimicrobial on NE*CHICKENS PER DAY	
79	Incubated Pathogenic Clostridium Perfringens Infected Chickens	Chickens
	= INTEG (infecting of pathogenic Clostridium perfringens susceptible chickens-developing of clinical NE infected chickens -developing of subclinical NE infected chickens-harvesting incubated pathogenic Clostridium perfringens	

	infected chickens -incubated pathogenic Clostridium perfringens chickens coinfected with coccidiosis, 0)	
80	Infected Chickens	Chickens
	= Clinical Coccidial Infected Chickens +Clinical NE and Coccidiosis Coinfected Chickens +Clinical NE Infected Chickens +Subclinical Coccidial Infected Chickens +Subclinical NE Infected Chickens	
81	infecting of Eimeria spp. susceptible chickens	Chickens/day
	= Coccidiosis effective contacts*Coccidiosis infectivity*Go end day	
82	infecting of pathogenic Clostridium perfringens susceptible chickens	Chickens/day
	= NE effective contacts*NE infectivity*Go end day	
	The rate at which birds are becoming infected with NE.	
83	Initial susceptible population to Clostridium perfringens	Chickens
	= (DELIVERY SIZE-Initial susceptible population to Eimeria spp.)*PULSE TRAIN (1, 1, Cycle period, FINAL TIME)	
84	Initial susceptible population to Eimeria spp.	Chickens
	= SAMPLE IF TRUE (Cycle day=1, Random Susceptibility*DELIVERY SIZE, 0)	
85	Initial total weight	Kg
	= DELIVERY SIZE*DAY OLD CHICK BODY WEIGHT*KG PER GRAM	
86	Litter Age	Cycle
	= INTEG (litter cycles-litter age reset, 1)	
87	litter age reset	Cycle/day
	= (1/TIME STEP)*(Litter Age-1)*PULSE TRAIN (LITTER REMOVAL FREQUENCY*Cycle period*PER CYCLE, 1, LITTER REMOVAL FREQUENCY *Cycle period*PER CYCLE, FINAL TIME)	
	Based on litter removal frequency, the litter age is reset to 1	
88	litter cycles	Cycle/day
	= 1*PULSE TRAIN (Cycle period, 1, Cycle period, FINAL TIME)	
89	NE effective contacts	Chickens/day
	= Fraction of NE infected chickens*NE susceptible contacts*Effect of antimicrobial on NE	
	The number of NE Susceptible Contacts that contact *Cl. perfringens* infected birds	
90	NE infectivity	Dmnl
	= NE infectivity for cycle*Effect of Feed composition	
	The proportion of effective contacts that will become infected with *Cl. perfringens* which is influenced by the proportion of birds infected with coccidia	
91	NE infectivity for cycle	Dmnl
	= SAMPLE IF TRUE (Cycle day=1, Random NE infectivity, 1e-05)	
	This maintains the randomly generated infectivity rate for NE through the cycle.	
92	NE start day	Days

	= NE start day for cycle	
93	NE start day for cycle	Days
	= SAMPLE IF TRUE (Cycle day=1, Random NE start day, 1e-05)	
94	NE susceptible contacts	Chickens/day
	= CONTACT RATE*Susceptible Chickens to Pathogenic Clostridium Perfringens	
95	Need for anticoccidial drugs administration	1
	= IF THEN ELSE (Anticoccidial Drugs Administration>0, 1, 0)	
96	Need for antimicrobial administration	1
	= IF THEN ELSE (Antimicrobial Administration>0.1, 1, 0)	
97	Percentage of clinical coccidiosis mortality	Dmnl
	= XIDZ (Clinical Coccidial Infected Chickens +Clinical NE and Coccidiosis Coinfected Chickens, Grow Out Chickens, 0)	
98	Percentage of clinical NE morbidity	Dmnl
	= XIDZ ((Clinical NE Infected Chickens +Clinical NE and Coccidiosis Coinfected Chickens), Grow Out Chickens, 0)	
99	Proportion chickens with coccidial protective immunity	Dmnl
	= XIDZ (Incubated Eimeria spp. Infected Chickens*EFFICACY OF VACCINE ADMISTRATION, Susceptible Chickens to Eimeria spp., 0)	
100	Proportion coccidial infected chickens	Dmnl
	= XIDZ (Total Live Coccidia Chickens, Total Live Chickens, 0)	
	The prevalence of birds infected with coccidiosis	
101	Proportion NE infected chickens	Dmnl
	= XIDZ(Total Live NE Chickens, Total Live Chickens, 0)	
102	Random coccidiosis infectivity	Dmnl
	= RANDOM Normal (0, 1, MEAN COCCIDIOSIS INFECTIVITY, STDDEV COCCIDIOSIS INFECTIVITY, SEED)	
103	Random coccidiosis start day	Days
	= RANDOM Normal (1, 11, MEAN COCCIDIOSIS START DAY, STDDEV COCCIDIOSIS START DAY, SEED)	
104	Random effective coccidial fatality	Dmnl
	= RANDOM Normal (0, 1, MEAN COCCIDIAL CASE FATALITY, STDDEV COCCIDIAL CASE FATALITY, SEED)	
105	Random effective NE and coccidial fatality	Dmnl
	= RANDOM Normal (0, 1, MEAN NE AND COCCIDIAL CASE FATALITY, STDDEV NE AND COCCIDIAL CASE FATALITY, SEED)	
106	Random effective NE fatality	Dmnl
	= RANDOM Normal (0, 1, MEAN NE CASE FATALITY, STDDEV NE CASE FATALITY, SEED)	
107	Random NE infectivity	Dmnl
	= RANDOM Normal (0, 1, MEAN NE INFECTIVITY, STDDEV NE INFECTIVITY, SEED)	
108	Random NE start day	Days

	= RANDOM Normal (1, 27, MEAN NE START DAY, STDDEV NE START DAY, SEED)	
109	Random subclinical coccidiosis fraction	Dmnl
	= RANDOM Normal (0, 1, MEAN SUBCLINICAL COCCIDIOSIS FRACTION, STDDEV SUBCLINICAL COCCIDIOSIS FRACTION, SEED)	
110	Random subclinical NE fraction	Dmnl
	= RANDOM Normal (0, 1, MEAN SUBCLINICAL NE FRACTION, STDDEV SUBCLINICAL NE FRACTION, SEED)	
111	Random Susceptibility	Dmnl
	= RANDOM Normal (0, 1, MEAN SUSCEPTIBILITY, STDDEV SUSCEPTIBILITY, SEED)	
112	Ratio of animal/soybean protein in feed	Dmnl
	= FEED ANIMAL PROTEIN CONTENT/FEED SOY PROTEIN CONTENT	
113	Ratio of NSP/corn in feed	Dmnl
	= FEED NSP CONTENT/FEED CORN CONTENT	
114	Recovery rate of coccidiosis	1/day
	= 1/COCCIDIOSIS INFECTIOUS PERIOD	
115	Recovery rate of NE	1/day
	= 1/NE INFECTIOUS PERIOD	
116	removing clinical coccidial dead chickens	Chickens/day
	= (Clinical Coccidiosis Dead Chickens/TIME STEP) *PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
117	removing clinical NE and coccidial coinfected dead chickens	Chickens/day
	= (Clinical NE and Coccidiosis Coinfected Dead Chickens/TIME STEP) *PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
118	removing clinical NE dead chickens	Chickens/day
	= (Clinical NE Dead Chickens/TIME STEP) *PULSE TRAIN (GO PERIOD, 1, Cycle period, FINAL TIME)	
119	Start day for anticoccidial drugs administration	Dmnl
	= IF THEN ELSE (Percentage of clinical coccidiosis mortality>= COCCIDIOSIS MORBIDITY TRIGGER, 1, 0)	
120	Start day for antimicrobial administration	Dmnl
	= IF THEN ELSE (Percentage of clinical NE morbidity>=NE MORBIDITY TRIGGER,1, 0)	
121	starting administration of anticoccidial drugs	1/day
	= IF THEN ELSE (PERIOD OF ANTICOCCIDIAL DRUGS ADMINISTRATION=0, 0, abs (Anticoccidial Drugs Administration-1)*Starting days for anticoccidial drugs administration*1/TIME STEP)	
122	starting antimicrobial administration	1/day
	= IF THEN ELSE (PERIOD OF ANTIMICROBIAL ADMINISTRATION=0, 0, abs (Antimicrobial Administration-1) *Starting days for antimicrobial administration *1/TIME STEP)	
123	Subclinical Coccidial Infected Chickens	Chickens

	= INTEG (developing of subclinical coccidial infected chickens + transforming of clinical coccidial to subclinical coccidial infected chickens-chickens infected with subclinical coccidiosis coinfected with NE-harvesting subclinical coccidial chickens, 0)	
124	Subclinical coccidiosis fraction	Dmnl
	= Subclinical coccidiosis fraction for cycle	
125	Subclinical coccidiosis fraction for cycle	Dmnl
	= SAMPLE IF TRUE (Cycle day=1, Random subclinical coccidiosis fraction, 1e-05)	
126	Subclinical NE Infected Chickens	Chickens
	= INTEG (developing of subclinical NE infected chickens + transforming of clinical NE to subclinical NE infected chickens-chickens infected with subclinical NE coinfected with coccidiosis-harvesting subclinical NE chickens, 0)	
127	Subclinical NE fraction	Dmnl
	= Subclinical NE fraction for cycle	
128	Subclinical NE fraction for cycle	Dmnl
	= SAMPLE IF TRUE (Cycle day=1, Random subclinical NE fraction, 1e-05)	
129	Susceptible Chickens to Eimeria spp.	Chickens
	= INTEG (chickens become susceptible to Eimeria spp. -harvesting susceptible chickens to coccidiosis -infecting of Eimeria spp. susceptible chickens, 0)	
130	Susceptible Chickens to Pathogenic Clostridium Perfringens	Chickens
	= INTEG (chickens become susceptible to Clostridium Perfringens-harvesting NE susceptible chickens -infecting of pathogenic Clostridium perfringens susceptible chickens, 0)	
131	Total Live Chickens	Chickens
	= Total Live Coccidia Chickens + Total Live NE Chickens + Susceptible Chickens to Eimeria spp. + Susceptible Chickens to Pathogenic Clostridium Perfringens + Clinical NE and Coccidiosis Coinfected Chickens	
132	Total Live Coccidia Chickens	Chickens
	= Incubated Eimeria spp. Infected Chickens + Subclinical Coccidial Infected Chickens + Clinical Coccidial Infected Chickens	
133	Total Live NE Chickens	Chickens
	= Incubated Pathogenic Clostridium Perfringens Infected Chickens + Subclinical NE Infected Chickens + Clinical NE Infected Chickens	
134	transforming of clinical coccidial to subclinical coccidial infected chickens	Chickens/day
	= DELAY1(Clinical Coccidial Infected Chickens*Fraction of recovered chickens from clinical coccidiosis to subclinical coccidiosis, Recovery Rate of coccidiosis)*CHICKENS PER DAY	
135	transforming of clinical NE to subclinical NE infected chickens	Chickens/day
	= DELAY1(Clinical NE Infected Chickens*Fraction of recovered chickens from clinical NE to subclinical NE, Recovery Rate of NE)*CHICKENS PER DAY	

136	transporting chickens to processing plants	Chickens/day
	= (Harvested Chickens/TIME STEP) *PULSE TRAIN (GO PERIOD+1, 1, Cycle period, FINAL TIME)	
137	Weight of clinical coccidiosis infected chickens	Kg
	= Average Weight per day*(1-WEIGHT LOSS OF CLINICAL COCCIDIOSIS INFECTED CHICKENS) *Clinical Coccidial Infected Chickens*KG PER GRAM	
138	Weight of clinical NE and coccidiosis coinfected chickens	Kg
	= Average Weight per day*Clinical NE and Coccidiosis Coinfected Chickens*(1-WEIGHT LOSS OF CLINICAL NE AND COCCIDIOSIS COINFECTED CHICKENS) *KG PER GRAM	
139	Weight of grow-out chicken	Kg
	= Average Weight per day*(Grow Out Chickens-Infected Chickens-Dead Chickens) *KG PER GRAM	
140	Weight of subclinical coccidiosis infected chickens	Kg
	= Average Weight per day*(1-WEIGHT LOSS OF SUBCLINICAL COCCIDIOSIS INFECTED CHICKENS) *Subclinical Coccidial Infected Chickens*KG PER GRAM	
141	Weight of subclinical NE infected chickens	Kg
	= Average Weight per day*(1-WEIGHT LOSS OF SUBCLINICAL NE INFECTED CHICKENS) *Subclinical NE Infected Chickens*KG PER GRAM	

Endogenous variables: factors in a causal model or causal system under study whose value is determined by the states of other variables in the system.

App.3 Abbreviations of terms

AGPs	Antibiotic growth promoters
A_w	Water activity
CLDs	Causal loop diagrams
CPH	*Cl. perfringens* associated hepatitis
FCR	Feed conversion ratio
HIV	Human immunodeficiency virus
NE	Necrotic enteritis
Nested PCR	Nested polymerase chain reaction
NSPs	Non-starch polysaccharides
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
SD model	System dynamics model
SIR model	Susceptible-infectious-recovered/removed model
SNE	Subclinical necrotic enteritis