Epidemiology of *Eimeria acervulina* infections in broilers

an integrated approach

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Lisette Graat

Proefschrift

ter verkrijging van de graad van doctor
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Graat, E.A.M. Epidemiology of *Eimeria acervulina* infections in broilers: an integrated approach (Epidemiologie van *Eimeria acervulina* infecties in vleeskuikens: een geïntegreerde benadering).

Understanding of factors that influence the epidemiology of *Eimeria acervulina* infections was increased by combined theoretical, experimental and field work. First, a simulation model was developed. Second, principles and phenomena as observed in simulation results were validated by conducting experiments in broilers. The research described in this thesis was focused on (qualitative) validation of the simulation model. The model was, in contrast to expectation, relatively insensitive to the sporulation rate of oocysts. Host immunity and anticoccidial drug efficacy, however, influenced model outcomes substantially. These aspects were tested experimentally. Simulation results showed existence of an optimal initial contamination level. This hypothesis was also tested in an experiment. From a qualitative perspective the simulation model behaves realistically. Quantitative agreement between simulation and experimental results was less satisfactory, which illustrates the need for better calibration of parameters and change of relationships in the current model. Finally, environmental and management factors that are associated with coccidiosis were studied using field data.

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STELLINGEN

1. De algemeen aanvaarde mening dat in nat strooisel oöcysten van *Eimeria* spp. beter sporuleren dan in droog strooisel, wordt in onderhavig onderzoek niet bevestigd.
   (Dit proefschrift)

2. Het risico op coccidiose in een mestronde van vleeskuikens is 2 tot 11 keer vergroot, wanneer in de vorige mestronde coccidiose is opgetreden. Een schoon begin is dus minstens het halve werk. *(Dit proefschrift)*

3. Stelling 2 impliceert dat minstens de helft van het geheim van het onder controle houden van coccidiose een goede hygiëne betreft. Wanneer een maximale hygiëne niet haalbaar is, bestaat het andere deel uit een optimaal infectieniveau. *(Dit proefschrift)*

4. Wanneer de immuuncompetentie van het vleeskuiken verminderd is, kan bij een zeer lage infectiedruk van *Eimeria acervulina* een betere weerstand tegen de parasiet worden opgebouwd dan bij een "normale" immuunstatus. *(Dit proefschrift)*

5. Het is onmogelijk efficiënt modelmatig onderzoek uit te voeren zonder een stevige basis in experimenteel werk.

6. Beleidsmatige beslissingen zouden meer wetenschappelijk ondersteund moeten worden in plaats van genomen te worden op basis van publieke opinie of consumentengedrag alleen.

7. Zowel voor het bereiden van een maaltijd als het uitvoeren van een experiment is een goed recept geen garantie voor succes.

8. Het in eerste instantie afleiden van de mate van volwassenheid van iemands lengte, heeft reeds veel mensen doen krimpen.

9. Een bad is heilzaam voor het lichaam, een soap voor de geest.

10. De maatschappelijke druk tot afschaffing van neusringen in de veehouderij lijkt ongegrond gezien de populariteit van piercings.

Stellingen behorend bij het proefschrift:
"Epidemiology of Eimeria acervulina infections in broilers: an integrated approach".
E.A.M. Graat
Wageningen, 13 december 1996

Aan Piet
Aan Papa & Mama

De berg is zwaar,
maar de vlinder tilt de kat op.

Voorwoord

Het is zover. Na ruim 4 jaar onderzoek op weg naar "De tijd is verstreken". Nu wordt het tijd om iedereen die me geholpen heeft, te bedanken.

Allereerst, de drie begeleiders "binnenshuis". Prof.dr. J.P.T.M. Noordhuizen, beste Jos, bedankt voor de vrijheid, het vertrouwen en de steun die je me onvoorwaardelijk gegeven hebt. Dit mag misschien cliché lijken, maar jij weet zelf wel beter. Dr.ir. A.M. Henken, beste André, de term "binnenshuis" was/is voor jou misschien minder van toepassing. Echter, in het E-mail tijdperk kunnen afstanden overbrugd worden waardoor jouw goede ideeën toch altijd goed terecht kwamen. Dr.ir. H.W. Ploeger, beste Harm, ik kon je altijd storen om van gedachten met je te wisselen, zelfs op je vrije dagen. Ook de snelheid waarmee je kritisch naar mijn manuscripten keek was ongekend. Jij was een belangrijke stimulans voor mij.

Verder, de leden van de begeleidingscommissie "buitenshuis": dr. W.W. Braunius, drs. M.H. Vertommen, drs. P.N.G.M van Beek, dr.ir. D.L. Kettenis, en dr.ir. A.A. Dijkhuizen. Bedankt voor jullie bereidheid zitting te nemen in de begeleidingscommissie en jullie waardevolle bijdragen aan proefopzetten en manuscripten.

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Velen hebben hun steentje bijgedragen bij klusjes op/bij het lab, bloedtappen, slachten van de dieren, de proefaccommodatie, de klimaat-respiratiecellen en de dataverwerking: Ger de Vries Reilingh, Mike Nieuwland, Frits Rietveld, Roel Terluin, Jan Veldhuis, Koos van der Linden, Marcel Heetkamp, Lenny van der Kooij, Klaas Frankena en alle studenten en stagiaires. Allemaal bedankt!

Plezier tijdens en naast het werk is ook essentieel. Bij mijn paranimfen, Truus Gijsbertse en Carla Wetzels, kon ik altijd mijn ei kwijt of gezellig kletsen, binnen en buiten de Zodiac muren. Ik vind het geweldig dat jullie tijdens de promotie aan mijn zijde willen staan. Ook wil ik mijn vakgroepsgenootjes bedanken voor m.n. de gezelligheid tijdens de koffie- en lunchpauzes.

Familie en vrienden, bedankt voor de interesse en afleiding. Papa en Mama, bedankt voor jullie ondersteuning als wij het weer eens te druk hadden. Anne-Marie, ik ben erg blij met de mooie omslag van mijn proefschrift.

Voor sommige mensen zijn woorden overbodig, hè Johan?

Lisette

Contents

General introduction

General Introduction

COCCIDIOSIS: A PROBLEM?

Poultry coccidia cause problems all over the world. Coccidiosis is an infectious disease caused by protozoa of the species *Eimeria* and has negative effects on the growth and feed efficiency of commercially reared broilers. The infection in the chicken starts with the intake of sporulated oocysts. After being ingested, excystation takes place and sporozoites are released from the sporocysts before invading intestinal cells. Subsequently, asexual and sexual multiplication results in excretion of oocysts with faeces. Outside the host, sporogony occurs, resulting in infectious oocysts and a new host can be infected (Current *et al.*, 1990). The oocysts of the parasite are very resistant to environmental influences and difficult to destroy with disinfectants. They can remain infectious for long periods of time, at least long enough to be carried on to the next flock cycle (Horton-Smith *et al.*, 1940; Reyna *et al.*, 1983). Even in especially designed isolation facilities coccidiosis outbreaks occur (Ovington *et al.*, 1995).

To prevent economic losses due to negative effects on production, anticoccidial drugs are used continuously. World wide sale of anticoccidials for broilers only is estimated at around \$300 million dollars annually (McDougald, 1990), indicating the economic importance of coccidiosis. Despite standard use of anticoccidial drugs in the chicken's diet, losses due to coccidiosis in intensively reared chickens are enormous and are approximately 2.2% of slaughter value (Braunius, 1987). Current losses are estimated at 1,000,000,000 (one billion) dollars annually in the world (Danforth & Augustine, 1990). This is, amongst others, caused by parasite resistance to anticoccidial drugs (Chapman, 1993).

However, even without occurrence of drug resistance there might be problems. These problems occur during the withdrawal period before slaughter, which can be up to 10 days. During this time period, coccidial infection in chickens may occur and results in damage which cannot be compensated for in the remaining time. These problems may be especially serious in case of very effective anticoccidial drugs because they do not allow development of protective immunity, which might be necessary in the withdrawal period. In broiler breeding or in replacement egg-laying flocks anticoccidials are used at suboptimal (less than recommended) levels to stimulate immunity formation without losses in production. This can also be done with usage of "older" less-effective drugs, or with application of step-down programmes in which the dosage of the drug is decreased gradually during the rearing period (Shirley *et al.*, 1995). This is done to allow protective immunity to build up. The degree of immunity after exposure to primary infections varies between different *Eimeria* species. Most immunogenic are the species *E. maxima* and *E.*

brunetti. E. acervulina, E. mitis, and *E. praecox* are moderately immunogenic, and *E. tenella* and *E. necatrix* are the least immunogenic (Ovington *et al.*, 1995). So, problems arise in determining the right dosage of an anticoccidial for a good balance between infection and build up of protective immunity against all species (Shirley *et al.*, 1995). This strategy is even more difficult in broilers due to the short life span, which is *e.g.* 5 to 6 weeks in The Netherlands.

CONTROL OF COCCIDIOSIS

Development of new drugs is very costly and therefore hinders such development (Aycardi, 1989). Also, the human population becomes more and more repulsive to the continuous use of drugs in diets of animals kept for human consumption, because of possible residues in the animal products (Aycardi, 1989; Tarbin *et al.*, 1993). Much research is focused on attempts to induce protective immunity and to understand mechanisms of it, and with that trying to develop an effective vaccine (Lillehoj & Trout, 1993). Protective immune responses can be activated with virulent or attenuated parasites. Critical point with virulent vaccines is the risk of severe infection (Shirley, 1992). This is especially valid for broiler production, in which effects of severe infection cannot be completely compensated for in the short life-span of the animals. Use of attenuated lines results in low oocyst reproduction and virulence, but it stimulates protective immune response (Lillehoj & Trout, 1993). This is very costly, because attenuated oocysts have to be produced in a large number of live animals. Moreover, it is very difficult to give birds the adequate dose, especially when it is administered through drinking water. Until now it is proved to be only cost-effective in broiler breeder flocks, not in broiler production.

Maternal immunisation is possible, but, it only may be effective the first 3 weeks at maximum (Smith *et al.*, 1994). When infection occurs at or after that moment in a flock cycle, the negative effects of production can not be compensated before the end of the grow-out in broilers (Voeten *et al.*, 1988).

There is some evidence of difference in susceptibility for coccidiosis between breeds and of a significant influence of the host genetic background on the development of protective immunity in young chickens (Mathis *et al.*, 1984; Lillehoj, 1988; Lillehoj & Trout, 1993). So, selection for resistant hosts is another possibility. However, resistance to one disease might lead to a higher susceptibility for other diseases and might be negatively correlated with production traits (Pinard, 1992).

Today, the diets of chickens consist generally of well grinded ingredients and are high in energy and low in fibre. This may attribute to atrophy and malfunctioning of the

gizzard and probably to a worse first defense to coccidiosis (Cumming, 1992). The malfunctioning digestive organs also might play a role in other diseases, which also may interact with coccidiosis. Changing diets can lead towards a decrease in negative effects of coccidiosis as shown by Allen *et al.* (1996).

Housing systems in which contact with faeces is not possible (for example wired floors) might be helpful in controlling the coccidiosis problem. However, considering animal welfare and regulations concerning housing systems, this is not a true solution.

STUDY OBJECTIVE

After summarising the problems with coccidiosis in current control strategies, and lack of information and limitations of alternative strategies, it can be concluded that there is a need for a way to control the negative effects of coccidiosis infection instead of eradication of the parasite. Therefore, knowledge about factors influencing introduction, course and spread of coccidiosis is needed. This refers to epidemiology which is the study of the natural occurrence of disease. The occurrence of an infectious disease is dependent on factors and processes that affect transmission and maintenance of disease agents. Both, host and parasites are influenced by a variety of factors (Scott, 1994). Mathematical models might be helpful in determining important influencing factors before doing costly experimental work.

Considering the problems in the control of coccidiosis, a research project was started to model *Eimeria acervulina* infections in broilers. The objective was to increase understanding of factors which influence the dynamics and mechanisms of an *Eimeria* infection in broilers and its effect on production, with combined theoretical, and experimental and field work. This was done according to the cycle of a modelling process (Figure 1) described by Kettenis (1990). First, a model was developed. Through experimentation with the model it was determined which experiments had to be done with the system (*i.e. E. acervulina* infection in broilers) to validate principles and phenomena found in simulation results. The main objective of the research project described in this thesis was focused on (qualitative) validation of the simulation model. Ideally, the project should contribute in the possibility to evaluate and/or support management decision strategies with regard to controlling coccidiosis and determination of their effects.
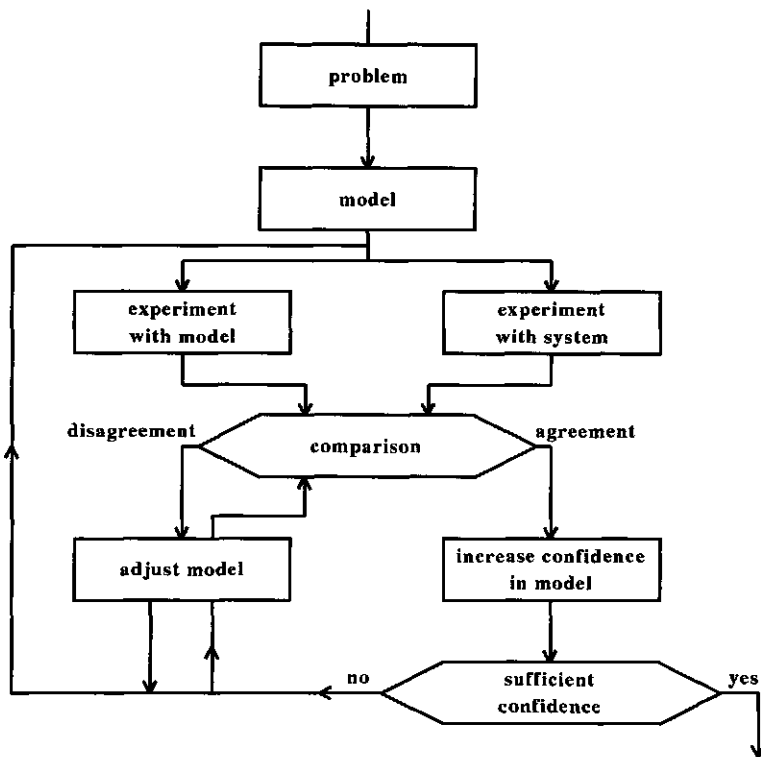
Figure 1. Cycle of modelling process (After: Kettenis, 1990).

THESIS OUTLINE

This thesis consists of 3 parts. The research done in Part I, formulating and using a computer simulation model, gave rise to hypotheses to be tested in experimental and observational work, which is described in Parts II and III.

Part I describes the simulation model, especially the population dynamics of the parasite *Eimeria acervulina* (Chapter 1.1), and effects of the parasite on broiler production (Chapter 1.2). Furthermore, a sensitivity analysis was done to observe parameters to which the model is sensitive (Chapter 1.3).

Most emphasis was placed on Part II, which deals with experimental validation of the model. This regards validation of parameters and phenomena which are regarded as very important according to expert's opinion (Chapter 2.1) or which were missing or less known when building the model and which turned out to be important in the sensitivity

analysis. Part II further describes qualitative validation of the model as a whole (Chapter 2.2) and of parts of the model (Chapter 2.3 to 2.5). This approach suits well in demonstrating principles or phenomena. Demonstration of these principles forms the leading theme of part II.

Furthermore, in the simulation model it was assumed that infection with *Eimeria acervulina* occurs in every flock. Data from poultry practice were analysed to investigate what the actual prevalence is and which factors influence the occurrence in a flock cycle (Chapter 3).

In the General Discussion the results are discussed with respect to the initial objective. Aspects for further research are considered.

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PART I

A simulation model of coccidiosis

Description of a simulation model for the population dynamics of *Eimeria acervulina* infection in broilers

A.M. Henken, H.W. Ploeger, E.A.M. Graat, T.E. Carpenter

Description of a simulation model for the population dynamics of *Eimeria acervulina* infection in broilers

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ABSTRACT

A simulation model for the population dynamics of *Eimeria acervulina* infection in broilers is presented. The model describes the development of the numbers of parasites in the various life stages during the growing period of broilers and the empty house period between grow-outs. The model includes assumptions with respect to development of immunity to *E. acervulina* infection and effects of application of anticoccidial drugs. The model consists of a set of difference equations that are solved numerically at 1 h intervals. Under constant conditions, an equilibrium level was reached after a few grow-outs during which infection always peaked around the 21st day in the growing period. Within a growing period, infection peaked earlier (later) than the 21st day in case initial numbers of sporulated oocysts were higher (lower) than the equilibrium number.

Key words: simulation, *Eimeria acervulina*, coccidiosis, anticoccidial drug efficacy, host immunity, broilers

INTRODUCTION

Coccidiosis is an infectious disease caused by protozoa of various *Eimeria* species. Five *Eimeria* species have been found in broilers, *Eimeria acervulina* (Tyzzer, 1929), alone or in mixed infection with *E. maxima* (Tyzzer, 1929) and/or *E. tenella* (Railliet & Lucet, 1891), being the most prevalent one (McDougald *et al.*, 1986; Braunius, 1987; Voeten, 1987). The disease became a significant problem when poultry meat production was intensified (Reid, 1990). Coccidiosis is combated preventatively by continuous application of drugs in the feed. Because of this application, clinical coccidiosis does not occur frequently. However, subclinical coccidiosis is present in almost every flock. Effects of subclinical coccidiosis are decreased rate of body weight gain and increased feed to gain ratio. There-

fore, in spite of advances in chemotherapy, management, nutrition and genetics, coccidiosis remains one of the most expensive and common diseases of poultry production (McDougald & Reid, 1991). Identification of factors affecting economic loss due to coccidiosis would require much experimentation and observation. Such research is costly and time consuming because many factors may be involved. A modelling approach might, therefore, be advantageous (Dijkhuizen, 1988; Sørensen & Enevoldsen, 1992). A model could be useful in identifying factors possibly involved and thus might reduce the amount of experimental and field research required. The basic model to which such factors could be added would have to cover three areas: (1) the population dynamics of the parasite; (2) the production characteristics of the host; (3) a financial summary of flock production allowing decision-making with respect to management practices in the next cycle. In this paper a simulation model for the population dynamics of *E. acervulina* is described. This species was chosen as it is the most prevalent cause of subclinical coccidiosis in broilers in practice.

MATERIAL AND METHODS

General

In The Netherlands, broilers are raised from hatching to slaughter weight in 44 days at maximum. Between successive flock cycles (grow-outs), a broiler house is empty for about 1-3 weeks. This empty period may arbitrarily be subdivided into a 'dirty' and a 'clean' period of about 2 and 11 days, respectively. The dirty empty period is the period immediately following delivery of the flock to the slaughter house. In this period, litter and equipment are taken out of the house. The next period, defined as the clean empty period, is the period wherein the house is thoroughly cleaned, disinfected, littered with fresh material, equipped and further prepared to receive chicks for the next flock cycle. In order to describe the population dynamics of an *Eimeria* infection each of the three periods mentioned, *i.e.* clean empty period, flock cycle, and dirty empty period, respectively, should be dealt with.

Although *E. acervulina* is a protozoon and therefore considered to be a microparasite, it is modelled as macroparasite because of the nature of its life-cycle and because of the quantitative relation between degree of infection and level of production of the host (Braunius, 1987; Anderson & May, 1991; McDougald & Reid, 1991).

Clean empty period

The only parasite stages present in the clean empty period will be oocysts that remained after removal of litter and equipment during the preceeding dirty empty period.

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These oocysts will all be sporulated and thus infective because all have been excreted at least 48 h earlier. Representing the dynamics mathematically gives the following equation for the number of sporulated oocysts at each point in time during the clean empty period (see Table 1 for symbols used):

$$SO_{(t)} = (1 - \mu_1 - \delta_1) \times SO_{(t-1)} \tag{1}$$

Thus, the number of sporulated oocysts at time t ($SO_{(t)}$) is estimated by taking the number present at a previous point in time, represented by t-1, and subtracting those oocysts that died between the time points t-1 and t because of normal biological reasons (μ_1) or because of measures intentionally taken by the farmer to decrease their numbers (δ_1).

Table 1. Explanation of symbols used in equations.

Symbol used	Explanation
$SO_{(t)}$	Number of sporulated oocysts per broiler present at time t
$TR_{(t)}$	Number of trophozoites per broiler present at time t
$SCHI_{(t)}$	Number of first-generation schizonts per broiler present at time t
$SCHII_{(t)}$	Number of second-generation schizonts per broiler present at time t
$OOC_{(t)}$	Number of oocysts to be excreted per broiler present at time t
$NSO_{(t)}$	Number of unsporulated oocysts per broiler present at time t
μ_i	Mortality coefficient of parasite stage i* due to normal biological processes
δ_i	Mortality coefficient of parasite stage i* due to additional measures taken at the farm
$\alpha_{(t)}$	Proportion of sporulated and unsporulated oocysts present that is ingested at time t
β	Sporulation coeffient for not yet sporulated oocysts
m_i	Multiplication factor when going from stage i* - 1 to stage i*
n	Residence time in number of t units in each of the internal host parasite stages, *i.e.* as TR, SCHI, SCHII and OOC, respectively
$AC_{(t)}$	Efficacy of anticoccidial at time t at each of the four internal host transitions (from SO to TR, from TR to SCHI, from SCHI to SCHII, and from SCHII to OOC, respectively)
$I_{(t)}$	Immunity at time t preventing development of the next parasite stage at the four internal host parasite stages
r	Proportion of sporulated and unsporulated oocysts in the house removed with litter at end of dirty empty period

* i=1, $SO_{(t)}$ during the clean empty period; i=2, $SO_{(t)}$ during the flock cycle and dirty empty period; i=3, $TR_{(t)}$; i=4, $SCHI_{(t)}$; i=5, $SCHII_{(t)}$; i=6, $OOC_{(t)}$; and i=7, $NSO_{(t)}$ during flock cycle and dirty empty period.

Flock cycle

The life-cycle of the parasite used in the model when broilers are present, is shown in Figure 1. The equations (2-7) to calculate the numbers of each stage at each point in time are presented in Table 2. All parasite stages were expressed in terms of numbers of sporulated oocysts ingested. A description of the coccidian life-cycle is given by, among others, Kheysin (1972) and Fayer & Reid (1982).
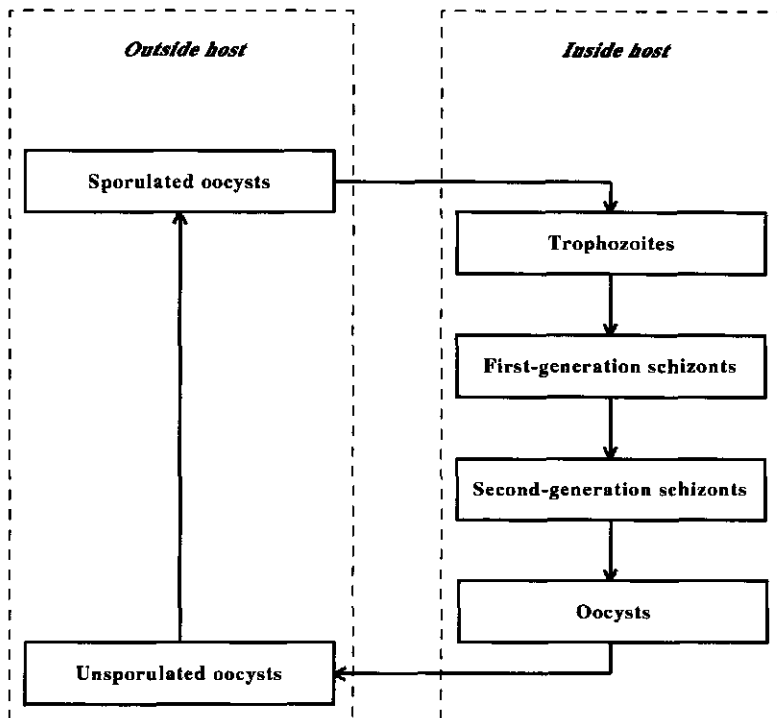
Figure 1. The life-cycle of *Eimeria acervulina* as used for modelling purposes.

Broilers ingest oocysts from a pool of sporulated oocysts in the house. After ingestion, the 8 sporozoites present in an oocyst (4 sporocysts with 2 sporozoites each) are released and penetrate intestinal epithelial cells and develop into trophozoites. Then, a phase of asexual multiplication (schizogony) begins wherein trophozoites develop into schizonts of the first-generation from which large numbers of first-generation merozoites are released. These first-generation merozoites penetrate new intestinal epithelial cells and develop into schizonts of the second-generation from which large numbers of second-generation merozoites are released. The phase of asexual multiplication might subsequently proceed for several more generations depending among others on the *Eimeria* species and strain involved. For modelling purposes, however, it is assumed that two schizont generations will suffice to represent asexual multiplication. Then, the merozoites of the second, and last, generation invade new cells wherein they develop into micro- or macrogametocytes entering a phase of sexual multiplication (gametogony) which ends with

the formation of zygotes that develop into oocysts to be excreted. At excretion, oocysts enter the pool of unsporulated oocysts from which they sporulate to enter the pool of sporulated oocysts. To allow the life-cycle to be completed, the host must be in contact with its excreta as is the case in broiler production.

Table 2. Equations (2) to (7) for calculation of the numbers of the various parasite stages ($SO_{(t)}$, $TR_{(t)}$, $SCHI_{(t)}$, $SCHII_{(t)}$, $OOC_{(t)}$, and $NSO_{(t)}$, respectively) present at each point in time during a flock cycle (see Table 1 for symbols)[*].

Life stage at time t	Staying in same stage	Entering new from previous stage	Leaving to next stage
$SO_{(t)}$	$(1-\mu_2-\delta_2) \times SO_{(t-1)}$	$\beta \times NSO_{(t-1)}$	$\alpha_{(t-1)} \times SO_{(t-1)}$
$TR_{(t)}$	$(1-\mu_3-\delta_3) \times TR_{(t-1)}$	$m_3 \times (1-I_{(t-1)}) \times (1-AC_{(t-1)}) \times \alpha_{(t-1)} \times SO_{(t-1)}$	$(1-\mu_3-\delta_3)^n \times m_3 \times (1-I_{(t-(n+1))}) \times (1-AC_{(t-(n+1))}) \times \alpha_{(t-(n+1))} \times SO_{(t-(n+1))}$
$SCHI_{(t)}$	$(1-\mu_4-\delta_4) \times SCHI_{(t-1)}$	$m_4 \times (1-I_{(t-1)}) \times (1-AC_{(t-1)}) \times (1-\mu_3-\delta_3)^n \times m_3 \times (1-I_{(t-(n+1))}) \times (1-AC_{(t-(n+1))}) \times \alpha_{(t-(n+1))} \times SO_{(t-(n+1))}$	$(1-\mu_4-\delta_4)^n \times m_4 \times (1-I_{(t-(n+1))}) \times (1-AC_{(t-(n+1))}) \times (1-\mu_3-\delta_3)^n \times m_3 \times (1-I_{(t-(2n+1))}) \times (1-AC_{(t-(2n+1))}) \times \alpha_{(t-(2n+1))} \times SO_{(t-(2n+1))}$
$SCHII_{(t)}$	$(1-\mu_5-\delta_5) \times SCHII_{(t-1)}$	$m_5 \times (1-I_{(t-1)}) \times (1-AC_{(t-1)}) \times (1-\mu_4-\delta_4)^n \times m_4 \times (1-I_{(t-(n+1))}) \times (1-AC_{(t-(n+1))}) \times (1-\mu_3-\delta_3)^n \times m_3 \times (1-I_{(t-(2n+1))}) \times (1-AC_{(t-(2n+1))}) \times \alpha_{(t-(2n+1))} \times SO_{(t-(2n+1))}$	$(1-\mu_5-\delta_5)^n \times m_5 \times (1-I_{(t-(n+1))}) \times (1-AC_{(t-(n+1))}) \times (1-\mu_4-\delta_4)^n \times m_4 \times (1-I_{(t-(2n+1))}) \times (1-AC_{(t-(2n+1))}) \times (1-\mu_3-\delta_3)^n \times m_3 \times (1-I_{(t-(3n+1))}) \times (1-AC_{(t-(3n+1))}) \times \alpha_{(t-(3n+1))} \times SO_{(t-(3n+1))}$
$OOC_{(t)}$	$(1-\mu_6-\delta_6) \times OOC_{(t-1)}$	$m_6 \times (1-I_{(t-1)}) \times (1-AC_{(t-1)}) \times (1-\mu_5-\delta_5)^n \times m_5 \times (1-I_{(t-(n+1))}) \times (1-AC_{(t-(n+1))}) \times (1-\mu_4-\delta_4)^n \times m_4 \times (1-I_{(t-(2n+1))}) \times (1-AC_{(t-(2n+1))}) \times (1-\mu_3-\delta_3)^n \times m_3 \times (1-I_{(t-(3n+1))}) \times (1-AC_{(t-(3n+1))}) \times \alpha_{(t-(3n+1))} \times SO_{(t-(3n+1))}$	$(1-\mu_6-\delta_6)^n \times m_6 \times (1-I_{(t-(n+1))}) \times (1-AC_{(t-(n+1))}) \times (1-\mu_5-\delta_5)^n \times m_5 \times (1-I_{(t-(2n+1))}) \times (1-AC_{(t-(2n+1))}) \times (1-\mu_4-\delta_4)^n \times m_4 \times (1-I_{(t-(3n+1))}) \times (1-AC_{(t-(3n+1))}) \times (1-\mu_3-\delta_3)^n \times m_3 \times (1-I_{(t-(4n+1))}) \times (1-AC_{(t-(4n+1))}) \times \alpha_{(t-(4n+1))} \times SO_{(t-(4n+1))}$
$NSO_{(t)}$	$(1-\mu_7-\delta_7 - \alpha_{(t-1)}) \times NSO_{(t-1)}$	$m_7 \times (1-\mu_6-\delta_6)^n \times m_6 \times (1-I_{(t-(n+1))}) \times (1-AC_{(t-(n+1))}) \times (1-\mu_5-\delta_5)^n \times m_5 \times (1-I_{(t-(2n+1))}) \times (1-AC_{(t-(2n+1))}) \times (1-\mu_4-\delta_4)^n \times m_4 \times (1-I_{(t-(3n+1))}) \times (1-AC_{(t-(3n+1))}) \times (1-\mu_3-\delta_3)^n \times m_3 \times (1-I_{(t-(4n+1))}) \times (1-AC_{(t-(4n+1))}) \times \alpha_{(t-(4n+1))} \times SO_{(t-(4n+1))}$	$\beta \times NSO_{(t-1)}$

[*] Each row of cells corresponds to one equation, going from equation (2) for $SO_{(t)}$ to equation (7) for $NSO_{(t)}$. Within each row, numbers are calculated as column 2 plus column 3 minus column 4.

The numbers of parasites in the various life stages present at a certain time is determined by: (1) the flow rate occurring in the cycle, whether this rate is constant or discontinuous due to time lags; (2) the multiplication factors between adjacent stages; (3) the prevention or reduction of flow between stages due to anticoccidials used; (4) the prevention of flow between stages due to immune responsiveness of the host; (5) mortality due to normal biological processes or due to measures intentionally taken by the farmer and not included in the former points. The numbers of parasites in a certain stage (Table 2, column 1) are quantified by taking the numbers that will stay in that stage (Table 2, column 2), adding those that newly enter that stage from the previous stage (Table 2, column 3) and subtracting those that continue to the next stage (Table 2, column 4).

Dirty empty period

During the dirty empty period after delivery of the broilers to slaughter, only sporulated and unsporulated oocysts remain. Mathematically these can be represented as (see Table 1 for symbols used):

$$NSO_{(t)} \quad = \quad (1 - \mu_7 - \delta_7 - \beta) \times NSO_{(t-1)} \tag{8}$$

$$SO_{(t)} \quad = \quad (1 - \mu_2 - \delta_2 + \beta) \times NSO_{(t-1)} \tag{9}$$

As soon as time has advanced to the end of the dirty empty period, the numbers of sporulated and unsporulated oocysts are calculated as (see Table 1 for symbols used):

$$NSO_{(t=end)} \quad = \quad (1 - r) \times (1 - \mu_7 - \delta_7 - \beta) \times NSO_{(t=(end-1))} \tag{10}$$

$$SO_{(t=end)} \quad = \quad (1 - r) \times (1 - \mu_2 - \delta_2 + \beta) \times NSO_{(t=(end-1))} \tag{11}$$

The estimate of remaining number of sporulated oocysts using equation (11) functions as initial environmental contamination level for the subsequent clean empty period (see equation 1) assuming that all unsporulated oocysts have sporulated or that those remaining are very small in number relative to the number of sporulated oocycts.

Parameter estimation

The parameter values adopted are summarized in Table 3.

Mortalities (μ_i and δ_i). Oocysts are well protected by their cell wall and may survive for long periods: survival periods of several months have been reported. Normal biological life expectancy of oocysts is estimated at 60 days (Braunius, 1987). The biological mortality rate used was therefore $\mu_1 = 1/(60 \times 24) = 0.0007/h$. Because of measures intentionally taken at a farm to decrease survival of oocysts, total mortality presumably will be at least twice the

normal biological mortality. Therefore, δ_1 was also set at 0.0007/h. During a flock cycle and during a dirty empty period life-expectancy of oocysts is considerably reduced in comparison to that in clean environments. Anaerobic conditions and ammonia production in litter with excreta are causes for this effect (Long & Rowell, 1975; Reyna *et al.*, 1983; Mathis *et al.*, 1984). In the model, normal biological mortality rate during the flock cycle and dirty empty period is assumed to be 5 times higher than in clean environments and similar for all parasite stages ($\mu_{i \neq 1} = 0.0035 (= 5 \times \mu_1)$). Also, in these conditions, additional mortality due to measures and/or specific farm conditions is assumed at least to equal normal biological mortality ($\mu_{i \neq 1} = \delta_{i \neq 1}$).

Table 3. Parameter values adopted in equations (1) to (11).

Parameter	Value adopted
μ_1 and δ_1	0.0007 *per capita* mortality/h
μ_2-μ_7 and δ_2-δ_7	0.0035 *per capita* mortality/h
β	0.0588 *per capita* sporulation/h
m_3	8 trophozoites/oocyst ingested
m_4	1 first-generation schizont/trophozoite
m_5	210 second-generation schizonts/fi rst-generation schizonts
m_6	105 oocysts to be excreted/second-generation schizont
m_7	1 excreted unsporulated oocyst/oocyst to be excreted
n	24 h residence in each of TR, SCHI, SCHII and OOC stage
r	in the dirty empty period 90% of the oocysts are removed with the litter and equipment (r=0.9)
$\alpha_{(t)}$	0.0008 to 0.008 *per capita* ingestion rate
$AC_{(t)}$	0 (in withdrawal period) to 75% prevention of upcoming of TR, SCHI, SCHII and OOC due to anticoccidial efficacy
$I_{(t)}$	5 (natural resistance) to 90% (maximal total immunity) prevention of upcoming of TR, SCHI, SCHII and OOC

Sporulation (β). Unsporulated oocysts are considered to sporulate at a rate of 0.0588/h which is based on an average sporulation time of 17 h (McDougald & Reid, 1991).

Multiplication factors (m_i). The reproduction index for *E. acervulina* amounts to 72000 (Brackett & Bliznick, 1952). This indicates that after correction for mortality of parasite stages and for effects of natural resistance of the host, about 72000 oocysts should be excreted per oocyst ingested in case no anticoccidials are used (AC=0; see equations in Table 2). The specific m_i can then be derived as follows: 1 oocyst will give rise to 8 trophozoites ($m_3 = 8$). Each trophozoite turns into one schizont ($m_4 = 1$). Each zygote turns into one oocyst ($m_7 = 1$). Moreover, the prepatent period for *E. acervulina* is 97 h. The level of natural resistance in a host, *i.e.* aspecific resistance in a host that never experienced an *E. acervulina* infection before, is set at 0.05 on a scale of zero to 1, the latter representing full protection. Then, $8 \times 1 \times m_5 \times m_6 \times 1 \times (1-0.0035-0.0035)^{97} \times (1-0.05)^4 = 72000$. Assuming first- and second-generation schizonts are equally productive and $m_6 = 0.5 \times m_5$, because m_6 is the

result of the combination of one macrogamete and at least one microgamete, then m_5 is about 210 and m_6 about 105 ($m_6=\sqrt{(72000/(2\times8\times0.5056\times0.8145))}$). The numbers for m_5 and m_6 are high, because the number of schizont generations was restricted to 2 while using the normal reproduction index of 72000/oocyst ingested.

Residence time in the various parasite stages (n). The pre-patent period for *E. acervulina* is reported to be 97 h. Assuming this period to be equally divided over the TR, SCHI, SCHII and OOC stage, then n is approximately 24 h.

Litter removal efficiency (r). It is assumed that with the litter the major proportion of sporulated and unsporulated oocysts will be removed from the broiler house. The constant r is therefore arbitrarily set at 0.9 on a scale from zero to 1, the latter meaning that all oocysts are removed with the litter.

Oocyst ingestion ($\alpha_{(t)}$). A certain proportion of the sporulated and unsporulated oocysts in the environment will be ingested by a host probably along with litter. This proportion is represented by $\alpha_{(t)}$ and is assumed to be related to feed intake as an indicator for foraging activity:

$$\alpha_{(t)} = \phi \times FI_{(t)}, \tag{12}$$

where, $\alpha_{(t)}$ =   proportion of oocysts ingested at time t,

   ϕ =   amount of oocyst intake/g of feed intake,

   $FI_{(t)}$ =   feed intake at time t.

It can be expected that α is small and laborious to estimate. It is, however, an important variable in the population dynamics of coccidiosis. In our companion paper on effects of coccidiosis on production characteristics (Henken *et al.*, 1994), it is shown that feed intake at a given time can be estimated from feed requirements for maintenance and body weight gain. These requirements for maintenance and body weight gain can be determined when body weight at a given time is known. Estimation of body weight in relation to age was done using a Gompertz growth curve (Ricker, 1979; Ricklefs, 1985; Zoons *et al.*, 1991). Feed intake was calculated to vary from about 0.52 g/h at young age to about 5.2 g towards the end of a 44 day grow-out period. Average daily gain would then be about 40 g with a feed to gain ratio of about 1.85. With a ϕ value set at 0.0015, $\alpha_{(t)}$ would vary from about 0.0008 to about 0.008/h and be within the range given by Parry *et al.* (1992). The latter are the only authors in the literature who reported a value for oocyst ingestion rate.

Anticoccidial efficacy ($AC_{(t)}$). Except for an obligatory pre-slaughter withdrawal period for some drugs, a broiler feed will always contain an anticoccidial drug under current intensive

management systems (McDougald, 1982). These drugs, however, do not often totally prevent development of intermediate parasite stages, since it was shown that immunity to ingested *Eimeria* species can develop despite drug usage. This phenomenon provides some protection during the drug withdrawal period towards the moment of delivery to the slaughter house. The mode of action is not known for all drugs. In the model it is assumed that anticoccidials are equally efficacious at each of the four internal parasite transitions, *i.e.* from oocyst ingested to trophozoite, from trophozoite to first-generation schizont, from first- to second-generation schizont, and from second-generation schizont to oocyst to be excreted. An anticoccidial efficacy of 75% was assumed, which means that at each of the four transitions only 25% of the potential number of the next stage evolves. Furthermore, it was assumed that after withdrawal of the anticoccidial from the feed, anticoccidial efficacy will continue to remain in full effect for 48 h more, after which it abruptly is set at zero. Withdrawal of anticoccidials was assumed to begin 5 days before slaughter.

Natural and acquired resistance $(I_{(t)})$. At the start of a flock cycle, chicks are immunologically unprotected to *Eimeria* infection except probably for some innate, natural, resistance level. Maternal immunity with respect to coccidiosis is considered to be unimportant in practice. Following *Eimeria* infection, specific disease resistance will develop dependent on the level of infection in time (Joyner & Norton, 1976). It is, however, assumed that in broilers this immunity will never reach the 100% level, meaning that full protection by immunity alone is not possible. It is also assumed that there will be no loss of immunity once it starts to develop or has developed. Although this may not be entirely true for all chickens (Rose, 1978, 1982) it seems to be a reasonable assumption for broilers because they are slaughtered at the relatively young age of about 44 days and exposure to *Eimeria* parasites in practice probably will be more or less continuous. Although not all internal host parasite stages may be equally immunogenic, the model uses at each point in time the summation of the cumulative numbers of new TR (=SCHI), SCHII and OOC as a measure of the amount of immunogen encountered (defined as CUMIM). Immunity was assumed to develop according to a growth function similar to the Gompertz equation for body weight development:

$$I_{(t)} = I_{max} \times exp[-ln(I_{max}/I_0) \times exp(-k_{(t)} \times t)], \tag{13}$$

where, $I_{(t)}$ =   immunity at age t with an initial value I_0,

I_{max} =   maximum attainable level of immunity as a proportion on a scale from zero to 1, the latter meaning total protection,

I_0 =   innate, natural, level of resistance,

$k_{(t)}$ =   rate of attainment of I_{max}.

The rate parameter $k_{(t)}$ in equation (14) can vary from k_{min} to k_{max} depending on CUMIM, the amount of immunogen encountered. When no infection occurs, specific immunity does not develop. In this case immunity will remain at the I_0 level. Then, if I_0 is assumed not to change with age as such, k_{min} can be set at zero. It is assumed that k_{max} is reached when CUMIM reaches a certain level. Beyond that CUMIM level, stimulation of immunity remains maximal at k_{max}. Then, the equation for $k_{(t)}$ becomes:

$$k_{(t)} = k_{max} \times (CUMIM_{(t\text{-lag})}/CUMIM_{(k=kmax)}), \tag{14}$$

where, $k_{(t)}$ = rate of attainment of I_{max} at age t, with the restriction that $k_{min} \leq k_{(t)} \leq k_{max}$,

k_{max} = k-value when $CUMIN_{(t\text{-lag})} \geq CUMIN_{(k=kmax)}$,

$CUMIM_{(t\text{-lag})}$ = summation of the cumulative numbers of TR (=SCHI), SCHII and OOC at age t-lag. If $CUMIM_{(t\text{-lag})} > CUMIM_{(k=kmax)}$ then $CUMIM_{(t\text{-lag})} = CUMIM_{(k=kmax)}$,

lag   = time delay between occurrence of parasite stage and its effect on $k_{(t)}$,

$CUMIM_{(k=kmax)}$ = the cumulative amount of internal host parasite stages at which $k_{(t)}$ becomes k_{max}.

The time lag between contact with immunogen and resulting effect on $k_{(t)}$ was assumed to be 5 days. The cumulative amount of internal host parasite stages at which $k_{(t)}$ becomes maximal was set at 20000. Other assumptions made were: $I_0 = 0.05$, $I_{max} = 0.9$ and $k_{max} = 0.0065$.

Initial conditions and calculations

At the start of simulation, the number of sporulated oocysts present in the house per broiler to be placed in the subsequent grow-out ($SO_{(t=0)}$) and the number of cycles to be simulated (CYCLES) must be provided. In the model, a cycle comprises an 11 day clean empty period, a 44 day flock cycle (grow-out), and a 2 day dirty empty period. The model solves the equations numerically at 1 h intervals proceeding from the beginning of the first clean empty period ($t=0$) to the end of the last dirty empty period ($t = CYCLES \times (11+44+2) \times 24$ h). The model was written in Turbo Pascal 6.0 (Borland International, Scotts Valley, CA, USA) on a IBM compatible computer.

To demonstrate the model, three levels of $SO_{(t=0)}$ were simulated. The choice of these levels was based on results of preliminary runs of the model over more than one cycle. These preliminary simulations showed that after a few cycles the number of SO with

which each subsequent cycle within the same run would start off with became constatn. This happended irrespective of the initial $SO_{(t=0)}$ provided to each simulation run, and is to be expected given that all conditions were kept constant for each flock cycle to be simulated within one run and enhanced by the fact that so far no stochastic processes are involved. The value of that resulting constant number of SO for each subsequent cycle was taken as the 'equilibrium' initial contamination number of oocysts provided to the model ($SO_{(t=0)} = 17$). This number was used as the basis to describe the results by providing it to the model and simulating one cycle and repeating this for a 100 times higher and a 100 times lower $SO_{(t=0)}$. In practice, changes will occur and decisions will be made from one cycle to the next. Consequently, it will be rare to find between subsequent flock cycles a constant initial contamination level. Therefore, we only show results for one flock cycle within each simulation run to demonstrate model behaviour in temporal numbers of parasite life-stages in dependence of the initial contamination level.
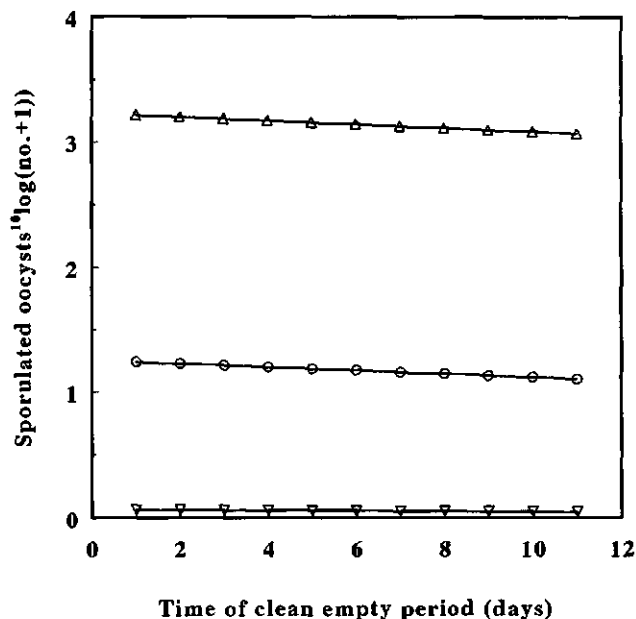
Figure 2. No. of sporulated oocysts per broiler to be placed in the next flock cycle during clean empty periods at the equilibrium (o), high (▵), and low (▿) initial contamination level.

R E S U L T S

During the clean empty period the number of sporulated oocysts declined due to natural (μ_1) and additional (δ_1) mortality. After 11 days, 69.1% of the original $SO_{(t=0)}$ remained which corresponds to $(1-0.0007-0.0007)^{(11\times24)} \times 100\%$ (Figure 2).

During the flock cycle, broilers ingested oocysts at a rate $\alpha_{(t)}$. At the equilibrium initial contamination level, the number of sporulated oocysts in the environment peaked at Day 21 of the flock cycle with a value of 100321 (Figure 3). For the high and low initial contamination levels the peak days with respect to number of sporulated oocysts were Day 13 and Day 27, respectively, with peak values of 117524 and 99676 (Figures 4 & 5). Numbers of unsporulated oocysts (NSO) peaked about 1.5 days before numbers of sporulated oocysts did (peak NSO numbers for equilibrium, high and low initial contamination levels were 31691, 39147, 36857, respectively). Numbers of SO and NSO at the high initial contamination level remained high for a longer period of time than those at the other contamination levels (Figure 4 *vs.* Figures 3 & 5). The oocyst ingestion rate increased over time except for a short period of time where growth rate and feed intake, were reduced by the *Eimeria* infection (Figure 6, equation 13). From the innate, natural resistance level, immunity increased sigmoidally (Figure 7). At the high initial contamination level, the amount of immunogen encountered rose more sharply than at the other initial contamination levels (Figure 4 *vs.* Figures 3 & 5). At this high level, immunity status developed according to the maximum rate from a relatively young age onwards.

Time of flock cycle (days)

Figure 3. No. of sporulated (———) and unsporulated (—— – –) oocysts, and cumulative number (– – – –) of new trophozoites, second-generation schizonts and oocysts to be excreted (CUMIM = new $(TR_{(t-i)} + SCHII_{(t-i)} + OOC_{(t-i)})$, i = start, end) per broiler during the flock cycle at the equilibrium initial contamination level $(SO_{(t-0)} = 17)$.
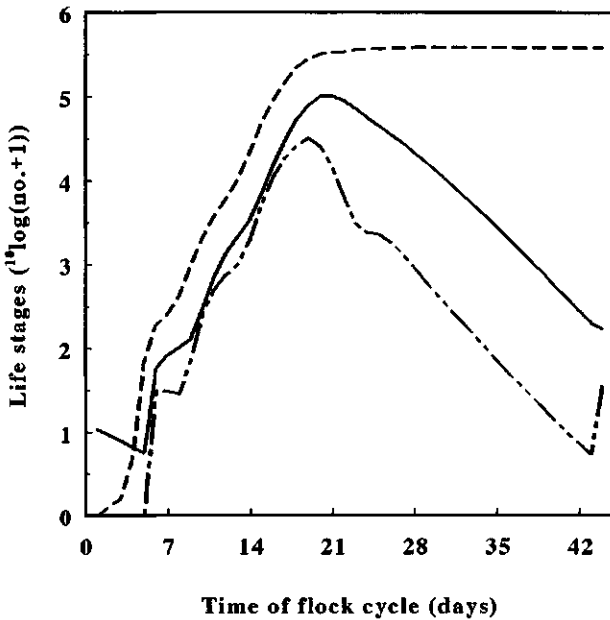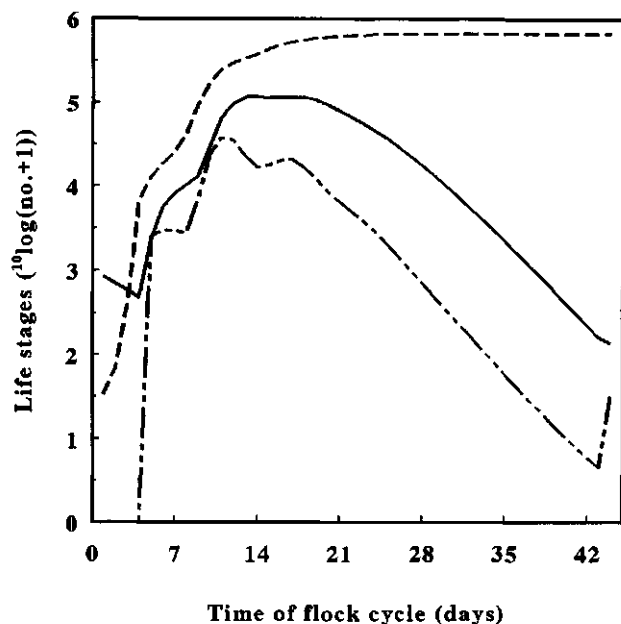
Figure 4. No. of sporulated (———) and unsporulated (—— – –) oocysts, and cumulative number (– – – –) of new trophozoites, second-generation schizonts and oocysts to be excreted (CUMIM = new $(TR_{(t-i)} + SCHII_{(t-i)} + OOC_{(t-i)})$, i = start, end) per broiler during the flock cycle at the high initial contamination level ($SO_{(t-0)} = 1700$).

At the beginning of the dirty empty period, sporulated as well as unsporulated oocysts remained in the house (Figure 8). Their numbers decreased over time, with a 90% drop at the end associated with removal of litter and equipment.

DISCUSSION

In the model it is assumed that sporulated oocysts always will be present in a broiler house. Their numbers, however, may vary with management practice. When broiler houses are cleaned and disinfected between cycles, as is done in The Netherlands, the initial number of oocysts probably will be low compared to situations where such management practices are not in use. There have been few quantitative studies of oocyst numbers in broiler house litter (Chapman & Johnson, 1992). Maximum oocyst numbers usually occur at 4-5 weeks of age in commercial broiler flocks followed by a decline (Long & Rowell, 1975; Reyna *et al.*, 1983). Time of peak numbers of oocysts may vary between *Eimeria* species. Oocyst numbers of *E. acervulina* peaked on average at Day 23 of the flock cycle,

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of *E. maxima* and mixed infections at Day 26, and of *E. tenella* at Day 29, using data from Dutch broiler farms (Braunius, 1987). The initial number of oocysts present will probably affect the chance of ingestion and thereby determine the timing of peak infection. This timing is important with respect to the amount of production depression (decreased body weight and worsened feed conversion), and thus economic loss, to be expected (Voeten, 1987). When the intention is to take advantage of the immune responsiveness of the host to combat the negative effects of coccidiosis, it may be advisable not to disinfect the environment to facilitate early exposure to the parasite (Fayer & Reid, 1982; Voeten, 1987; Reid, 1990). The advice to use less effective anticoccidial drugs at the beginning of a flock cycle is in line with this (Braem & Suls, 1992). It might, however, be that at early infection age may limit immune reactivity of the host (see Figure 7). As probably early infection is correlated with relatively heavy infection, decreased hygienic measures in the empty period may not be the advisable way to facilitate exposure, not even mentioning the risk of other disease outbreaks as well.
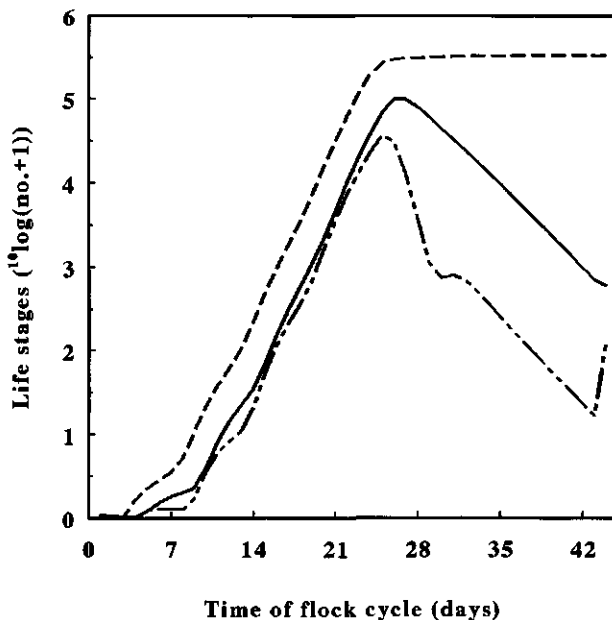
Figure 5. No. of sporulated (———) and unsporulated (—— - -) oocysts, and cumulative number (— — — —) of new trophozoites, second-generation schizonts and oocysts to be excreted (CUMIM = new (TR$_{(t=i)}$ + SCHII$_{(t=i)}$ + OOC$_{(t=i)}$), i = start, end) per broiler during the flock cycle at the low initial contamination level (SO$_{(t=0)}$ = 0.17).

The oocyst ingestion rate increased in time during a flock cycle because feed intake increased. When infection reached a certain level, defined by Henken *et al.* (1994) as the

production effect threshold level, feed intake fell temporarily to a lower level and, as a consequence oocyst ingestion decreased too. In practice, this may happen also at the end of a flock cycle in case long withdrawal periods are applied. Peak values of infection occurred because hosts mounted an immune response against the parasites. According to Long et al. (1975), three factors may contribute to a decrease in oocyst numbers in the litter of older birds: decreased excretion of oocysts due to development of immunity; increased mortality of oocysts due bacterial action and ammonia production; and suppression of oocyst production due to the effect of drugs. In our model, only immunity was varied in time while parasite mortalities, whether due to normal biological reasons, anticoccidial efficacy or additional measures, remained constant. It can be shown that at constant conditions, drug efficacy as such cannot be a reason for occurrence of peak oocyst presence: infection would steadily decrease or increase at constant immunity and mortality. In contrast to what is assumed in the model, oocyst mortality may increase with time due to changing ratio of excreta to litter as the birds grow older. Although anticoccidial efficacy as such may not cause a peak in infection, the balance between anticoccidial efficacy and immune potential (I_{max} and rate of attainment of I_{max}, see equation 14) is probably very important. When irregularities in one or both occur, the infection may increase uncontrollably (Graat et al., submitted).
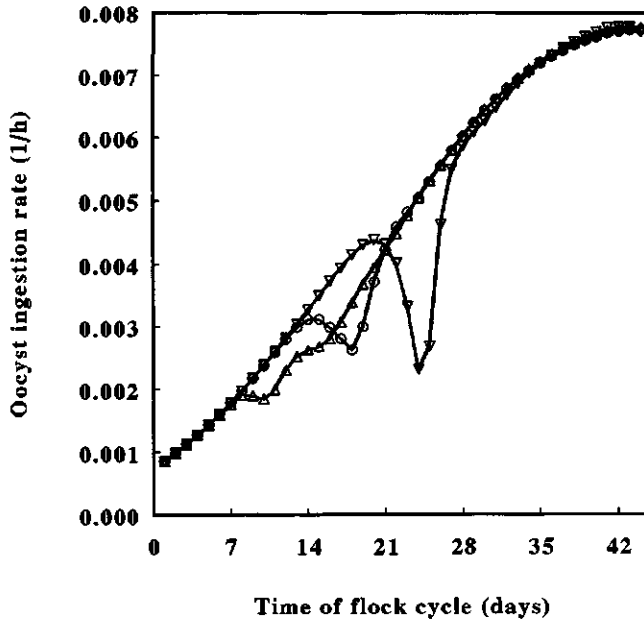

Figure 6. Oocyst ingestion rate during flock cycles at the equilibrium (o), high (Δ), and low (∇) initial contamination level.
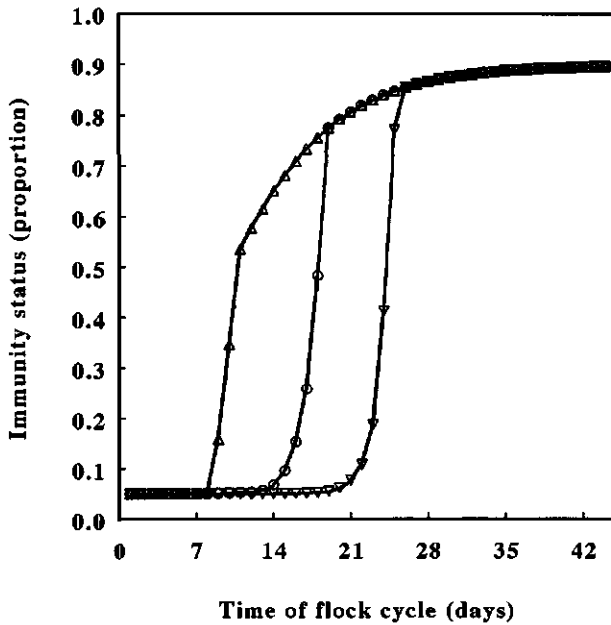
Figure 7. Immunity status of hosts during flock cycles at the equilibrium (o), high (∆), and low (∇) initial contamination level.

During the dirty empty period, model output for the low initial contamination level was somewhat above, and for the high initial contamination level, somewhat below that of the equilibrium level. When run over more cycles, the low and the high level would stabilize at the equilibrium level. Therefore, these initial deviations are considered to be caused by overcompensation *en route* towards equilibrium.

The present model was developed to serve as research tool. Models as such can be very effective in developing understanding of the ecology and population dynamics of parasite populations (see for example Anderson & May, 1991). Although the model presented does not, for instance include stochastic elements with respect to oocyst ingestion or immune reactivity, it will help focus future experimental and observational research on missing or less known important quantitative aspects of the life-cycle of the parasite (see Graat *et al.*, 1994) and its effects on production. As such, deterministic models are useful for determining the sensitivity of a system's behaviour to changes in certain parameters (Hurd & Kaneene, 1993). A sensitivity analysis of the present model to parameters related to the population dynamics of *E. acervulina* infection in broilers will be presented by Graat *et al.* (submitted).
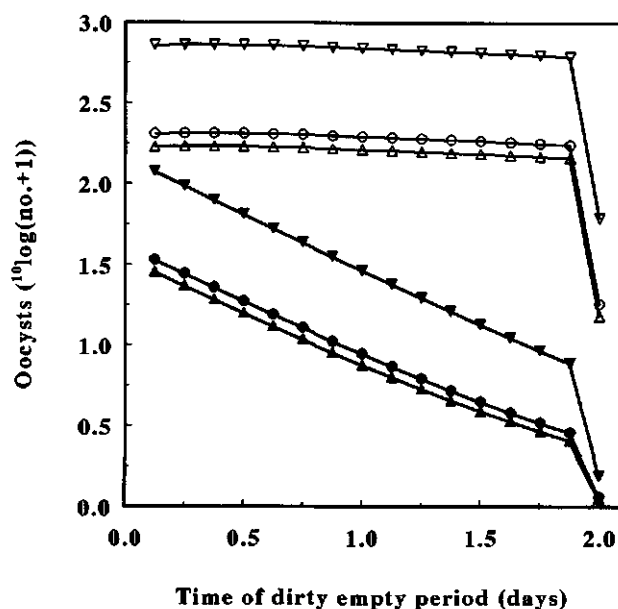
Figure 8. No. of sporulated (open symbols) and unsporulated (closed symbols) oocysts per broiler grown in the previous flock cycle during the dirty empty period at the equilibrium (o), high (∆), and low (∇) initial contamination level.

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Description of a model to simulate effects of
Eimeria acervulina infection on broiler production

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Description of a model to simulate effects of
Eimeria acervulina infection on broiler production

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ABSTRACT

A simulation model for effects of *Eimeria acervulina* infection on technical and economic characteristics in broiler production is presented. The model describes development over time of the growth depression, feed intake reduction, and decrease in feed efficiency associated with infection. The model also shows a phase of compensatory growth during which earlier negative effects are counterbalanced, at least partly. Major assumptions made were: infection with *E. acervulina* occurs in each flock; production is affected in each flock; compensatory growth takes place because immunity develops and cell regeneration occurs. The results show that the pattern of development of the production characteristics during a flock cycle depends on the initial contamination level. Both, a high and low initial contamination level results in a lower average daily gain, a worse feed to gain ratio, and a reduced net revenue compared to an intermediate contamination level.

Key words: simulation, *Eimeria acervulina*, coccidiosis, production characteristics, net revenue, broilers

INTRODUCTION

Coccidiosis is an infectious disease caused by protozoa of various *Eimeria* species. *Eimeria acervulina* (Tyzzer, 1929), alone or in mixed infection with *E. maxima* (Tyzzer, 1929) and/or *E. tenella* (Railliet & Lucet, 1891) is the most prevalent agent causing coccidiosis in broilers (McDougald *et al.*, 1986; Braunius, 1987; Voeten, 1987). Because of continuous application of drugs, clinical coccidiosis no longer occurs frequently. However, subclinical coccidiosis is present in almost all broiler flocks. In a companion paper (Henken *et al.*, 1994) a simulation model for the population dynamics of an *E. acervulina* infection in broilers was described.

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Occurrence of subclinical coccidiosis in broilers has large effects on production. Voeten (1987) reported that subclinical coccidiosis decreased net revenue per broiler, on average, by DFL 0.06 to 0.07 (1 DFL ≈ 0.56 US$), mainly due to a decreased rate of gain and worsened feed to gain ratio. Subclinical coccidiosis may increase the feed to gain ratio by 0.1 kg of feed/kg of body weight gain and may reduce body weight at the time of slaughter by 100 g (Voeten, 1989). Comparison of measures to combat subclinical coccidiosis should be based on the efficacy of those measures to reduce loss of production or, in economic terms, to reduce loss in net revenue. Ideally, such a comparison of efficacy should be done before a specific measure is chosen. To allow *a priori* comparison, the relation between the population dynamics of the parasite and its effect on the host should be known. In the present paper a theoretical model is presented that simulates the effects of an *E. acervulina* infection on production characteristics and net revenue.

MATERIAL AND METHODS

General

In The Netherlands, broilers are raised from hatching (about 40 g) to slaughter weight (about 1800 g) in 44 days at maximum with a feed to gain ratio of about 1.85. Between flock cycles (grow-outs), a broiler house is empty for about 1 to 3 weeks. The population dynamics of the parasite during grow-outs and empty periods were described by Henken *et al.* (1994), who assumed subclinical coccidiosis always to be present. A major assumption in the present paper is that beyond a certain level (threshold) of infection, production will be negatively affected. Moreover, beyond that level, the negative effect will be greater at higher infections. The production characteristics to be dealt with are: body weight development, feed intake, feed to gain ratio, and host mortality.

Body weight development

The development of body weight of broilers over time may be described by a Gompertz equation (Ricker, 1979; Ricklefs, 1985; Zoons *et al.*, 1991). This equation was rewritten to:

$$BW_{(t)} = BW_{(t=0)} \times \exp[RG_{(t)} \times (1 - \exp(-rg \times t))], \tag{1}$$

where, $BW_{(t)}$ = body weight at age t,

$RG_{(t)}$ = rate of attainment of mature weight,

rg = instantaneous rate of growth at the inflection point.

Parameter rg was found by dividing absolute daily body weight gain at the inflection point (about 60 g/d) by body weight at that time (about 1400 g)(rg = 0.043/day). Parameter $RG_{(t)}$ was calculated as:

$$RG_{(t)} = RG' \times (1/(1 + \epsilon \times (DC_{(t)} - DC_{threshold}))), \tag{2}$$

where, $RG_{(t)}$ =   rate of attainment of mature weight,

         RG' =   rate of attainment of mature weight without infection,

         ϵ =   effect of one DC on RG' above $DC_{threshold}$,

         $DC_{(t)}$ =   number of damaged cells at time t,

         $DC_{threshold}$ =   threshold level of DC beyond which growth rate will be reduced.

Equation (2) was used in case $DC_{(t)}$ was greater than $DC_{threshold}$, otherwise RG' was used for $RG_{(t)}$. Parameter RG' can be estimated as $\exp(rg \times t_g)$ where t_g represents the age of broilers at which the inflection point in the growth curve occurs (about 5 weeks). Consequently, a value of 4.5 was adopted for RG'. The parameter $DC_{(t)}$ was estimated by using the cumulative number of new evolving internal host-parasite stages, assuming that each new stage damages one cell (except when proceeding from trophozoites to first-generation schizonts), corrected for cell regeneration. By definition, without regeneration correction, this parameter is identical to CUMIM (cumulative amount of immunogen encountered) as used by Henken *et al.* (1994). So, $DC_{(t)}$ can be calculated as:

$$DC_{(t)} = (1 - \gamma) \times DC_{(t-1)} + NEW_t, \tag{3}$$

where, $DC_{(t)}$ =   number of damaged cells at time t,

         γ =   regeneration rate of DC,

         NEW_t =   new evolving damaged cells at t.

The parameter NEW_t was obtained from the model that simulates the population dynamics of *E. acervulina* (Henken *et al.*, 1994). The constant γ was set at 0.004/h, assuming that restoration of cell function takes about 10 days counted from the moment the specific cell was invaded by a parasitic life-stage. The constants $DC_{threshold}$ and ϵ were arbitrarily set at 1000 and 3×10^{-7}, respectively.

Feed intake and feed to gain ratio

Feed intake can be expressed as a function of metabolic body weight ($BW^{0.75}$), as

a measure of maintenance requirements for metabolisable energy (ME_m), and body weight gain (as a measure of requirements for production above maintenance (ME_p)). Coccidiosis may affect both maintenance and body weight gain. The influence of coccidiosis on body weight gain has already been defined through the rate parameter $RG_{(t)}$. The influence of coccidiosis on maintenance can be defined through an effect on increased need for nutrients due to leakage of body materials into the intestinal tract and extra requirement for increased tissue regeneration. Also, because of impaired digestion and absorption, the ability to metabolise feed may be decreased by infection, indicating that relatively more feed is needed for maintenance and gain compared to uninfected animals. The equation for feed intake can be described as:

$$FI_{(t)} = (a_{(t)} \times BW_{(t)}^{0.75} + c \times BWG_{(t)}) / ME\%_{(t)}, \qquad (4)$$

where, $FI_{(t)}$ = feed intake at time t,

$a_{(t)}$ = the amount of feed needed for the maintenance of 1 g of metabolic weight depending on the level of infection at time t,

$BW_{(t)}$ = body weight at time t,

c   = net feed conversion ratio,

$BWG_{(t)}$ = absolute body weight gain at time t,

$ME\%_{(t)}$ = ability to metabolise feed relative to uninfected animals.

The parameter $a_{(t)}$ may be represented by:

$$a_{(t)} = a' + (DC_{(t)}/WF_{MEm}), \qquad (5)$$

where, $a_{(t)}$ = amount of maintenance feed needed/gram of metabolic body weight,

a'   = constant, representing the amount of feed needed for the maintenance of 1 g of metabolic weight in the absence of infection,

$DC_{(t)}$ = number of damaged cells at time t,

WF_{MEm} = weighting factor for $DC_{(t)}$ to calculate its effect on a.

Calculation of a' is possible because maintenance requirements (about 480 kJ of ME/kg of metabolic body weight per day) and dietary energy density (13 kJ ME/g) are known (Thorbek & Henckel, 1976; Wenk & Van Es, 1976; National Research Council,

1984; Ketelaars *et al.*, 1986). Therefore, a′ is about 0.0084 g of feed/h/g of BW$^{0.75}$ (480/(24×13.4×1000$^{0.75}$)). Assuming that a$_{(t)}$ at heavy, but still subclinical, infections (DC$_{(t)}$=1×10^6) will be about 10% higher than a′, WF$_{MEm}$ was set at 12×10^8.

The constant c in equation (4) can be estimated using the energy density of body material (about 10 kJ/g) and of feed (about 13 kJ/g), and the net energetic efficiency with which dietary energy given above maintenance is converted to energy deposited as body tissue (about 0.65) (De Groote, 1974; National Research Council, 1984; Ketelaars *et al.*, 1986; Henry *et al.*, 1988). Therefore, c will be approximately 1.25.

The parameter ME%$_{(t)}$ in equation (4) was determined as:

$$ME\%_{(t)} = 1 - (DC_{(t)}/WF_{ME\%}), \tag{6}$$

where, ME%$_{(t)}$ = ability to metabolise feed relative to uninfected animals,
DC$_{(t)}$ = number of damaged cells at time t,
WF$_{ME\%}$ = weighting factor for DC$_{(t)}$ to calculate its effect on ME%.

Assuming that ME%$_{(t)}$ at heavy, but still subclinical, infections (DC$_{(t)}$=1×10^6) will be reduced by 10% at maximum, WF$_{ME\%}$ was set at 1×10^7.

At each point in time, feed to gain ratio was determined as cumulative feed intake divided by total body weight gain until that time.

Host mortality

It was assumed that subclinical coccidiosis associated with *E. acervulina* infection, does not affect broiler mortality. However, to determine revenues, the average mortality as occurring in broiler flocks (about 5% per grow-out) was taken into account. The number of live broilers at each point in time was calculated according to:

$$LB_{(t)} = LB_{(t=0)} \times \exp(-\mu_b \times t), \tag{7}$$

where, LB$_{(t)}$ = number of broilers alive at time t,
LB$_{(t=0)}$ = number of broilers placed in the house at the start of the flock cycle,
μ_b = broiler mortality rate.

The constant μ_b was set at 0.00005/h.

Costs and revenues

Net revenue was determined per flock assuming the simulated host to be representative for the flock average. Average flock size at placement was considered to be 16000 which is comparable to practice (Henken *et al.*, 1992). Factors taken into account and values adopted are presented in Table 1. The difference in net revenue of infected and also simulated uninfected controls was defined as the hypothetical loss occurring due to subclinical coccidiosis.

Table 1. Economic factors and their adopted values.

Factor	Value (in DFL[1])
Cost:	
Day-old chicks (per 100)	57
Feed with anticoccidia (per 100 kg)	60
Feed without anticoccidia (per 100 kg)	58
Litter (per chick)	0.030
Light, heating, water (per chick)	0.116
Veterinary costs (per chick)	0.060
Interest with respect to animals (per chick)	0.018
Picking and transport at delivery (per chick)	0.067
Gross revenue:	
Broilers delivered (per kg live weight)	1.70

[1] 1 DFL ≈ US$ 0.56

Initial conditions and calculations

The model was written in Turbo Pascal 6.0 (Borland International, Scotts Valley, CA, USA). Calculations were done at 1 h intervals. Henken *et al.* (1994) found that irrespective of initial level of oocyst contamination, an equilibrium number of oocysts was reached after a few simulated cycles. Results obtained at this level (called EQUILIBRIUM: $SO_{t=0} = 17$) were compared with those obtained at a 100 times higher (HIGH) and 100 times lower (LOW) initial contamination level. To obtain an estimate of the losses in production and revenue due to coccidiosis, an uninfected control group was also included.

RESULTS

The threshold level of number of damaged cells affecting growth rate, was reached on Day 9 at the equilibrium initial oocyst contamination level ($DC_{threshold} = 1000$, Figure 1). At the high (low) contamination level this threshold number was reached earlier (later), *i.e.* on Day 3 (16). Body weight gain of infected animals became lower than that of

uninfected broilers after $DC_{threshold}$ was reached (Figure 2). Maximum growth depression occurred on Days 16, 18, and 24, at the high, equilibrium, and low initial contamination levels, respectively. At the high initial contamination level an earlier, but smaller, dip in daily body weight gain was present on Day 11. At the low initial contamination level, the period of growth depression was more confined, but very much pronounced. After the depression in body weight gain, growth rate reached the level of that of uninfected hosts again after which growth compensation occurred (Figure 2). Due to this compensation the difference in body weight between uninfected and infected animals became smaller after having been maximal at about Day 29 (Figures 3 & 4).
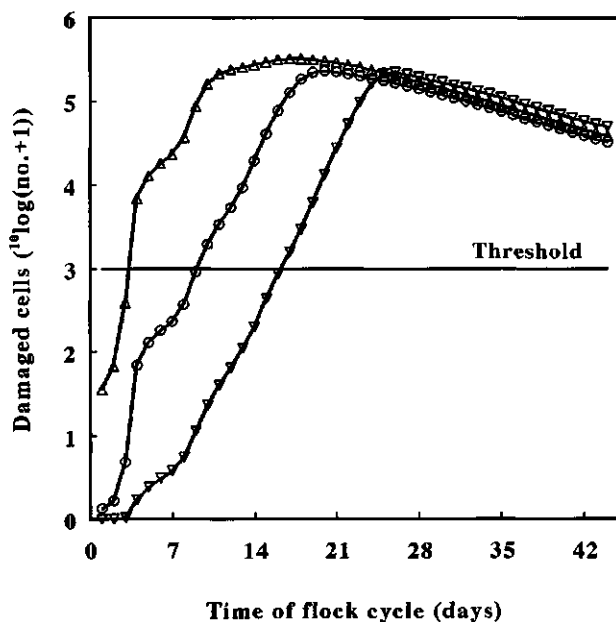
Figure 1. The number of damaged cells during flock cycles at the equilibrium (o), high (▵), and low (▿) initial contamination levels. The number of damaged cells above which daily body weight gain is affected is indicated also (Threshold).

As feed intake was derived from daily body weight gain, the patterns in both were similar. The cumulative feed to gain ratio is shown in Figure 5. Feed utilisation became less efficient compared to that of uninfected broilers from the moment body weight gain was falling behind. The technical and economic characteristics over the whole 44 day cycle are summarized in Table 2. Among the infected groups, average daily body weight gain was highest at the equilibrium initial contamination level. At this level also feed to gain ratio was lowest. Therefore, the hypothetical loss in net revenue was lowest at the equilibrium initial contamination level.

Figure 2. Daily body weight gain during flock cycles for uninfected (————) and infected broilers (at equilibrium (o), high (Δ), and low (▽) initial contamination levels).

Figure 3. Body weight during flock cycles for uninfected (————) and infected broilers (at equilibrium (o), high (Δ), and low (▽) initial contamination levels).

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Figure 4. The difference in body weight between uninfected and infected broilers (at equilibrium (o), high (Δ), and low (∇) initial contamination levels).

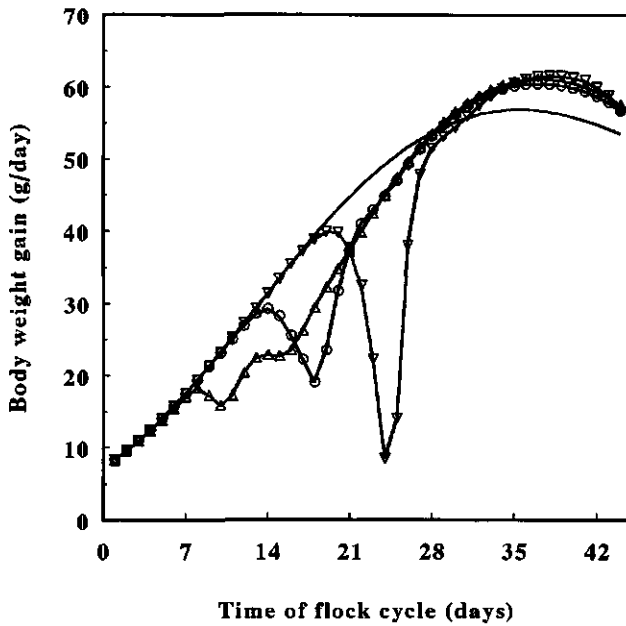**Figure 5.** Cumulative feed to gain ratio during flock cycles for uninfected (———) and infected broilers (at equilibrium (o), high (Δ), and low (∇) initial contamination levels).
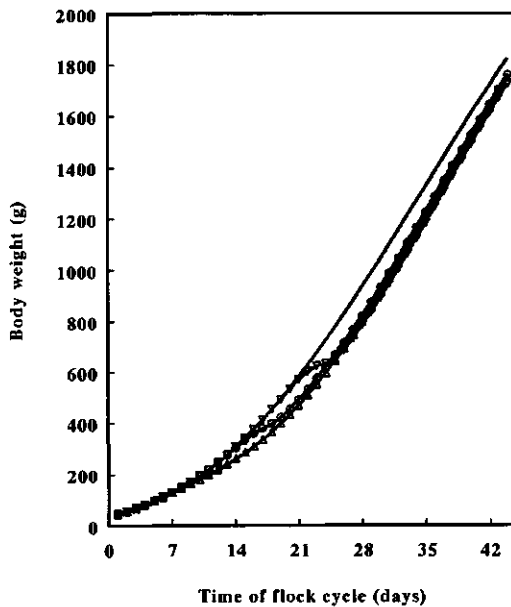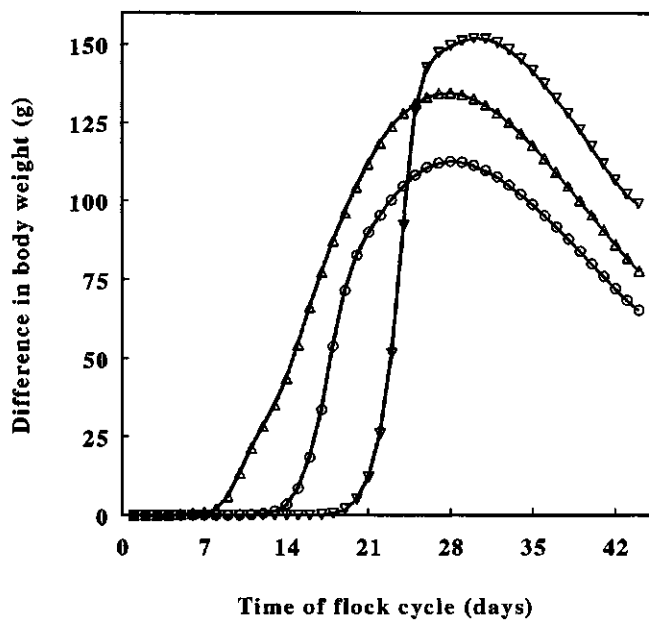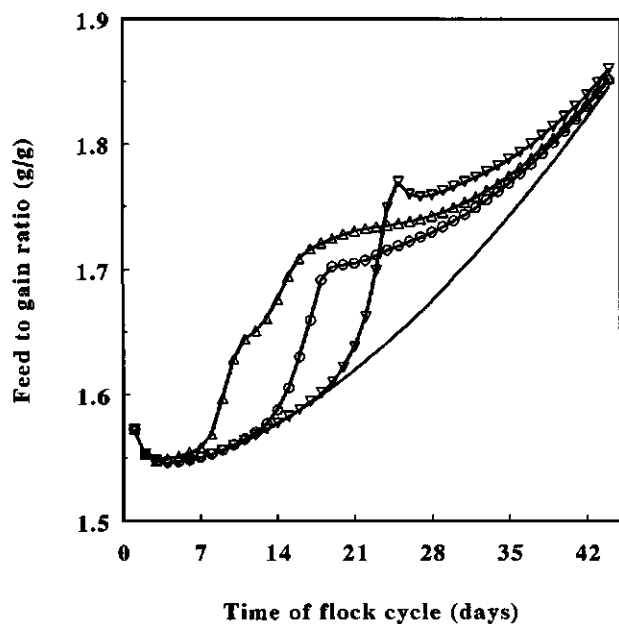
Table 2. Technical and economic characteristics for a 44-d simulated flock cycle.

Initial contamination level	Body weight at slaughter (g)	Average daily (g/d)	Feed: gain (g/g)	Net revenue[1] (cts/broiler)[2]
Equilibrium	1762	39.13	1.852	15.919
High	1749	38.84	1.853	15.123
Low	1728	38.36	1.860	13.046
Zero[3]	1827	40.61	1.846	20.103

[1] A 5% mortality was assumed at each contamination level.
[2] 1 ct = DFL 0.01 ≈ US$ 0.0056
[3] Uninfected controls

DISCUSSION

Henken *et al.* (1994) assumed that subclinical coccidiosis occurs in each flock of broilers. As a consequence, production will be affected in each flock at some time in the cycle. At early infection, compensation of production depression may occur later in the cycle (Voeten, 1987). The depression in feed intake and daily gain is temporary because immunity develops after contact with the parasite. Continuous exposure to low parasite levels is especially effective in stimulating the immune system (Joyner & Norton, 1976). After immunity has developed and cell regeneration has occurred, a phase of compensatory growth may offset, at least partly, the negative effects of *Eimeria* infection. However, on average, early infection will be synonymous with high initial contamination levels. The production depression at this high level may, therefore, persist for a longer period of time compared to lower contamination levels. At high initial contamination levels, compensatory growth will occur relatively late in the cycle. At low initial contamination levels, the depression in daily gain falls late in the cycle and the time for compensatory growth is limited. Also, at a low initial contamination level, the number of sporulated oocysts in the environment is still relatively high towards the end of the cycle. This may result in a new depression of daily gain in case long durg withdrawal periods are applied. An intermediate initial contamination level may be optimal for net revenue. This optimum level is not necessarily the equilibrium level simulated. In Figure 6 the relation between the hypothetical loss (difference in net revenue between uninfected and infected animals) and initial contamination level is presented. Loss was minimal at an initial contamination level that was about 4 times higher than the equilibrium level resulting from the present study. At this optimal contamination level numbers of sporulated oocysts peaked at Day 18 in the cycle compared to the peak at Day 21 at the equilibrium and Day 13 at the high initial contamination level (Henken *et al.*, 1994). It might therefore be that in an effort to reduce

loss by coccidiosis a point is reached beyond which the effect is just the opposite of that which is pursued. A less efficient disinfection in the empty period between cycles (Voeten, 1987), application of less efficient anticoccidia (Braem & Suls, 1992), or use of other means that would increase early exposure (artificial introduction of oocysts?) might well be advantageous. It should be realized that the quantitative outcome of the model simulations will depend largely on the assumptions made including the one concerning length of cycle. A major assumption is, of course, that a decrease in technical and economic results due to coccidiosis always occurs. Even in the most optimal case, the reduction in net revenue was still about DFL 0.036 per broiler, or 18%. The qualitative behaviour of the model will be relatively insensitive to the assumptions made indicating the existence of an optimal contamination level assuming that oocysts always will be present (Graat *et al.*, 1996).
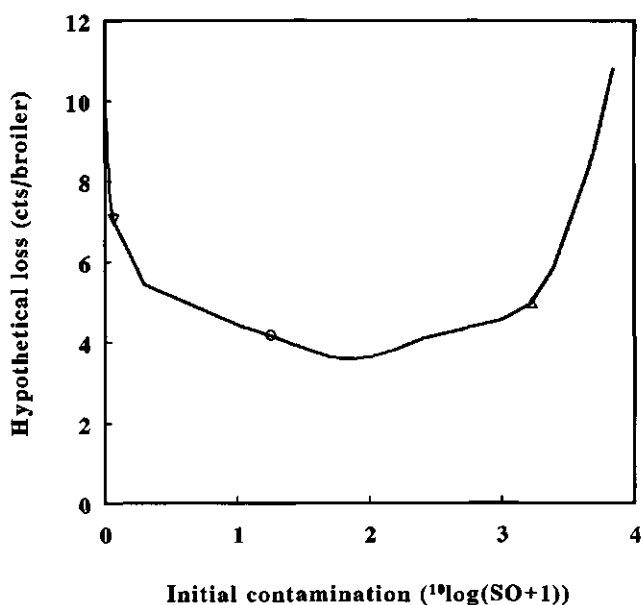
Figure 6. Hypothetical loss per broiler during a 44 day cycle in relation to contamination level in terms of numbers of sporulated oocysts (SO) initially present per broiler (SO > 0). For comparison reasons, the location of the equilibrium (o), high (Δ), and low (∇) initial contamination level is indicated along the curve as well.

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Sensitivity analysis of a model simulating population dynamics of an *Eimeria acervulina* infection in broilers and its subsequent effects on production and net revenue

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ABSTRACT

A sensitivity analysis on a simulation model for the population dynamics of *Eimeria acervulina* infection in broilers and its effects on technical and economic characteristics was done. The effect on body weight gain, feed to gain ratio, and net revenue of changing parameters of this deterministic model was determined. This sensitivity analysis indicated that the model is relatively insensitive to values of the parameters sporulation rate, mortality rate and intake rate of oocysts. Results showed that the model is especially sensitive to parameters related to anticoccidial drug efficacy and host immunity with respect to maximum attainable immunity, the rate at which maximum immunity is attained and time lag between parasite contact and development of protective immunity.

Key words: sensitivity analysis, simulation model, coccidiosis, *Eimeria acervulina*

INTRODUCTION

A deterministic model which simulates the population dynamics of an *Eimeria acervulina* infection in broilers and subsequent effects on production characteristics and net revenue has been described by Henken *et al.* (1994a,b). The main purpose of the model was to get a more detailed understanding of the parasitic life cycle and of the interaction of the parasite with its host, including effects of a coccidiosis infection on production traits and, hence, on net revenue. Since resistance to anticoccidial drugs is a major problem (Chapman, 1993) and development of a vaccine is very slow due to the complex nature of the parasite (Lillehoj & Trout, 1993), other ways have to be found to control the negative effects of infection. Modelling might reveal lacks in current knowledge and can be very useful to point out which experiments have to be done to learn more about the biological system (Baldwin, 1995).

The model contains about 40 parameters; some being constants and some varying with time or driven by some other variable. The value of several parameters is known fairly well. For other parameters educated guesses could be made, while for others sheer guessing was the only alternative, which is common for most models (Shannon, 1975). Prior evaluation, *i.e.* some validation of the model against data from an experiment, in which pattern of infection and its effect on production characteristics was observed, showed adequate qualitative model behaviour (Graat *et al.*, 1996).

This paper describes the results of a sensitivity analysis on the simulation model of Henken *et al.* (1994a,b). Besides validation, one of the most important concepts in simulation modelling is sensitivity analysis, *i.e.* determining model behaviour in response to changes in the values of parameters used (Shannon, 1975).

MATERIAL AND METHODS

The model in short

A simplified causal loop diagram underlying the model is presented in Figure 1. The model starts with the number of sporulated oocysts of *Eimeria acervulina* which remained from the previous flock. Only sporulated oocysts are infectious to the host. The number of infectious oocysts is influenced by litter removal efficiency. So, at the start of a flock cycle there is a number of sporulated oocysts in the litter and the parasitic life cycle can start through intake of oocysts by the host. Within the host the parasite multiplies and causes damage to epithelial cells of the intestines. The rate of intake of oocysts by the host influences the number of internal host parasite stages and with that the number of excreted oocysts. Rate of sporulation then determines the number of sporulated oocysts in the litter and one life cycle is completed. The number of internal host parasite stages influences development of immunity, which in turn controls the rate of multiplication of internal host parasite stages. This rate of multiplication is also influenced through application of anticoccidial drugs. In each step of the life cycle, mortality of parasite stages occur, which disappear through the "sink". The number of parasitic individuals influences production characteristics, such as broiler weight and feed efficiency and, hence, the economic profit, *i.e.* net revenue per broiler. Finally, when a broiler is infected, growth and feed intake are negatively influenced as well as intake rate of oocysts since this is related to feed intake.

Method

Not all parameters were examined, because some are driven by other variables. Hence, only parameters that are constants and parameters with initial starting values were

examined. Parameters were selected for the sensitivity analysis, either because data from literature were lacking or because an eminent effect on model outcome was presumed. Parameters investigated were intake rate of oocysts (α), sporulation rate of oocysts (ß), mortality rate of parasite stages (μ), anticoccidial drug efficacy (AC), and immunity status of the host (IMM) (Table 1). Immunity status was divided in several components: innate natural level of immunity (INNATE), maximum level of immunity attainable after exposure (MAXIMM), maximum development rate from innate to maximum immunity level (MAXGRO), time lag between parasite contact and effect on immunity (TIMELAG), and cumulative number of damaged mucosa cells of the intestines (CUMIMM) (Table 2). The cumulative number of damaged mucosa cells is assumed to be representative for the total amount of antigen encountered and thus involved in development of immunity. Default values of these model parameters are presented in Tables 1 & 2.

The simulation model (Henken *et al.*, 1994a,b) showed a parabolic relationship between initial contamination level, *i.e.* number of sporulated oocysts at start of the flock cycle, and net revenue. So, model parameters were investigated at three initial contamination levels (7 = BELOW, 70 = OPTIMUM, 700 = ABOVE oocysts per broiler), to include this parabolic relationship. Assuming that oocysts are always present in broiler houses, the optimum level is defined as the level at which economic loss is minimal. Coccidiosis occurs in up to 75% of all flocks (Braunius, 1988).
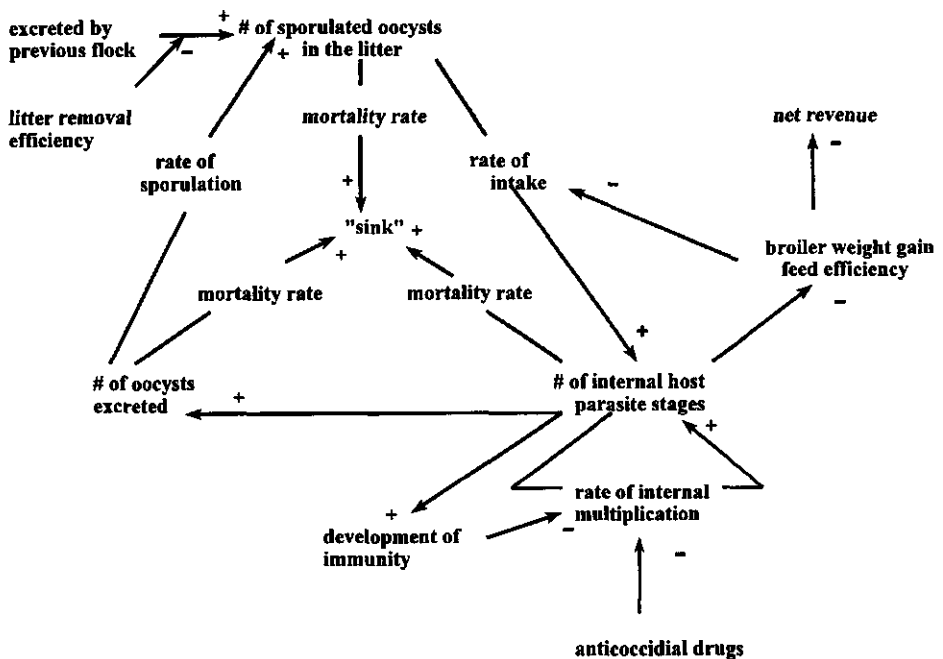

Figure 1. A simplified causal loop diagram underlying the simulation model (the symbol '+' corresponds with positive association, and '-' with a negative association).

Table 1. Default model parameter values.

Parameter		Default value
Intake rate of oocysts	α	0.0008 to 0.008 per h depending on feed intake
Sporulation rate	ß	0.0588 per h of unsporulated oocysts
Mortality rate of oocysts	μ	0.0014 per h in clean empty period, and 0.007 per h in flock cycle and dirty empty period
Immunity status of host	IMM	5% (natural resistance) to 90% (maximum total immunity) prevention of upcoming of internal parasite stages
Anticoccidial efficacy	AC	0 (in withdrawal period), 75% (rest of flock cycle)

Table 2. Default model parameter values of components of immunity status.

Parameter		Default value
Attainable immunity	MAXIMM	0.90 (maximum as a proportion of 1)
Basic level immunity	INNATE	0.05 (as a proportion of 1)
Immunity development rate	MAXGRO	0.0065 per h (maximum)
Damaged cells	CUMIMM	cumulative number of damaged cells with time lapse and a maximum of 20000 cells
Reaction time immunity	TIMELAG	120 h: time lag between parasite contact and build up of immunity

Model parameters were changed in turn to be proportionally (0.05, 0.10, 0.15, 0.20, 0.25) greater or less than their default value. This range was assumed to be equal to variability in real life. The sensitivity of the model to change in parameter values was assessed by its effect on simulated outcomes for body weight gain (BWG), feed to gain ratio (FGR) and net revenue (NR). The influence of immunity status on BWG, FGR and NR was examined from a proportional change until +10%, since immunity (MAXIMM) cannot exceed 1. For the most important parameters, as indicated above, a combination of changes was studied; this was the interaction between immunity status and anticoccidial efficacy. Results were subjected to analysis of variance to see which variables were of significant influence (SAS, 1989).

RESULTS

Results were parallel for all initial contamination levels. Therefore, only results of the optimum level are presented. The figures for the outcomes body weight gain, feed to gain ratio and net revenue were of similar pattern and therefore only figures of body weight gain will be shown throughout the results. Figure 2 shows the effect of percentage change in model parameters on body weight gain.
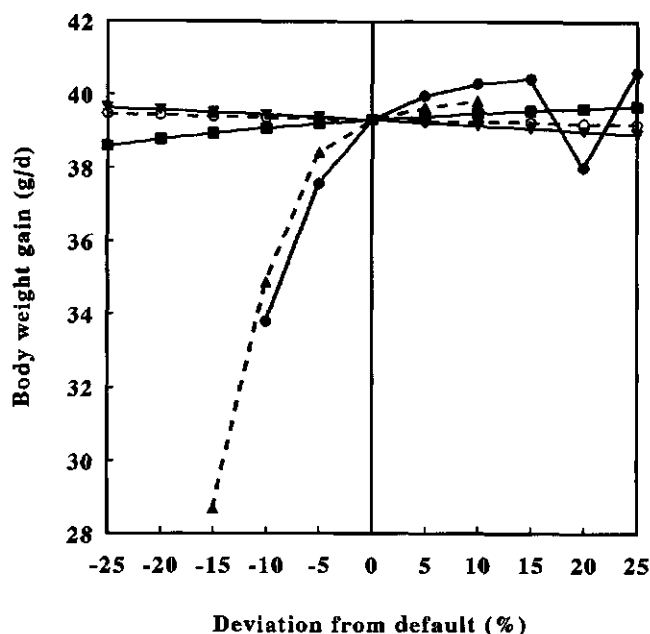
Figure 2. The effect of percentage changes from the default value in model parameters, anticoccidial efficacy (•——•), immunity status (▲ – – – ▲), intake rate of oocysts (▼———▼), sporulation rate of oocysts (o – – – o) and mortality rate of oocysts (■———■) on body weight gain for the optimum infection level.

Body weight gain, feed to gain ratio and net revenue improved slightly with a higher mortality rate of oocysts (μ), or a lower sporulation rate of oocysts (ß) or a lowered intake rate of oocysts (α). This improvement was not significant. However, the change in value of the parameters AC and IMM showed a significant effect ($P < 0.001$). A huge decrease in BWG, FGR and NR occurred when anticoccidial efficacy or host immunity was lowered. For anticoccidial efficacy, the model outcomes were only calculated until values up to -10% of its default value, and for immunity status until -15%. Lower values of the default led to unrealistic low results from a subclinical infection point of view. Furthermore, a significant interaction effect ($P < 0.001$) between anticoccidial efficacy and host immunity was present. Figure 3 shows this interaction effect on BWG. Not all combinations of IMM and AC are presented in this figure, because results of these combinations were also unrealistic low from a subclinical infection viewpoint.

The model became insensitive to changes in the level of immunity status (IMM) when anticoccidial efficacy (AC) was increased with at least 5%. Conversely, when IMM was increased with 5 or 10% the model became similarly insensitive to changes in AC. However, just a slight decrease in anticoccidial efficacy in combination with a lowered

immunity status had an enormous negative effect on production characteristics.

Since the parameter IMM had a significant effect on model outcomes, each of its components in the model (INNATE, MAXIMM, MAXGRO, TIMELAG, and CUMIMM) was investigated to detect which of these were most important. Figure 4 shows the effect of percentage change in parameters related to immunity status on body weight gain. With a lowered MAXIMM, BWG and NR worsened ($P<0.001$ and $P<0.001$, respectively), as well as with a lowered MAXGRO ($P<0.001$ and $P<0.001$, respectively), or increased TIMELAG ($P<0.001$ and $P<0.001$, respectively). FGR was only significantly ($P<0.001$) influenced by MAXIMM.
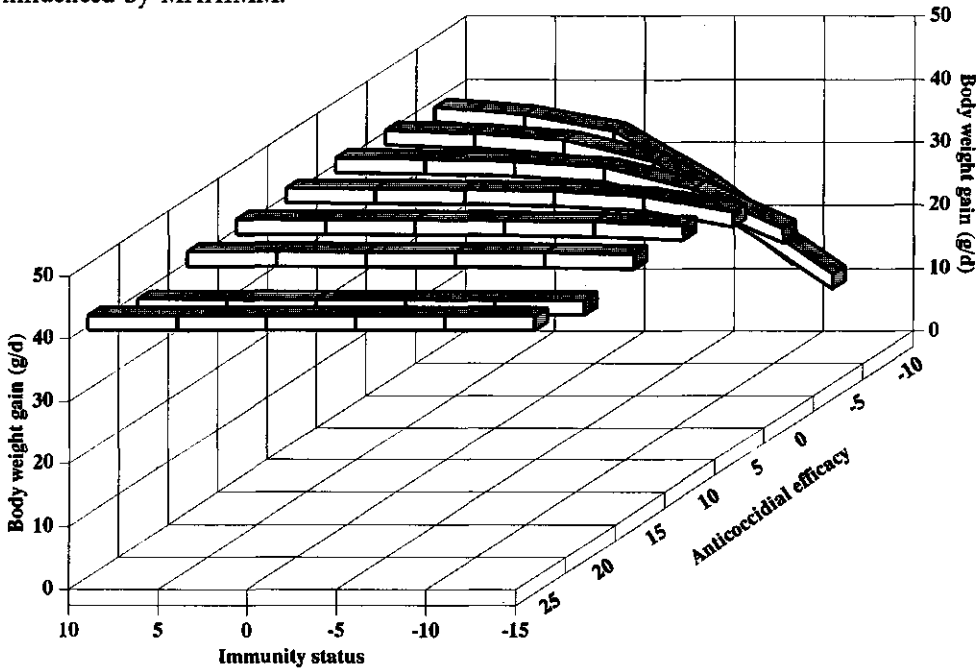
Figure 3. The effect of combinations of changes from the default value in parameters related to immunity status and anticoccidial efficacy on body weight gain for the optimum infection level.

DISCUSSION

Testing validity of the model has to be done by comparing predictions with results from experiments or field studies. When these agree over a wide range of different circumstances, confidence in the understanding of the system is obtained. If not, needs for additional or more reliable (experimental) data to estimate parameters more precisely are highlighted with a sensitivity analysis (Black *et al.*, 1993). Prior evaluation of the model

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with experimental data showed adequate qualitative behaviour (Graat *et al.*, 1996). On the other hand, Martin *et al.* (1987) stated that sensitivity analysis may be conducted to demonstrate the degree to which conclusions based on initial parameter values remain valid if the values used are not accurate estimates of the true value.
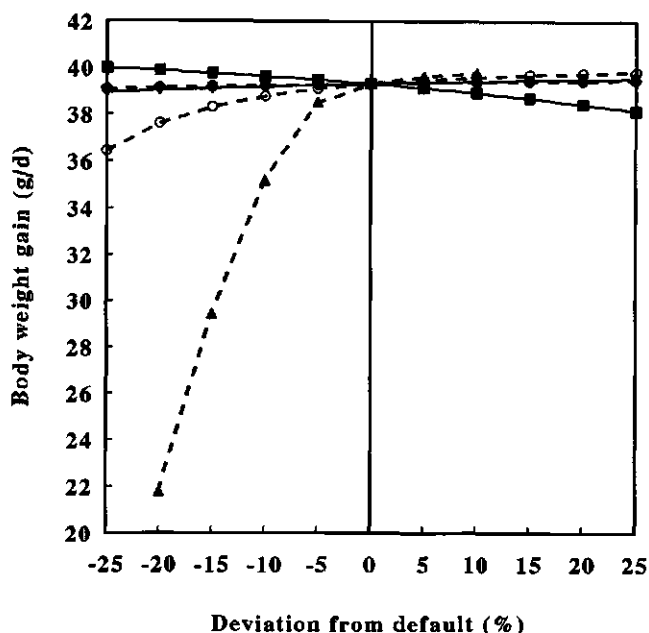
Figure 4. The effect of percentage changes from the default value in model parameters which are components of immunity status, basic level of immunity (● – – – ●), maximum attainable immunity (▲ – – – ▲), cumulative number of damaged cell of mucosa of intestines (▼———▼), immunity development rate (o – – – o), and time lag between parasite contact and build up of immunity (■———■) on body weight gain for the optimum infection level.

Expected variability in real life was induced in this study by changing parameter values within a range of 25%. However, examined changes may not occur at all in poultry practice. Although sensitivity analysis may indicate importance of a parameter it may not be important in real life situations since it might be biologically irrelevant. On the other hand some default values have such a low value that a change of 100 or even 200% may be more appropriate. Changes in parameters related to mortality rate, intake rate and sporulation rate of oocysts did not change model outcomes much. However, in the model, a sporulation rate of 0.0588 results approximately in 83% of all oocysts sporulating. Experiments showed that in litter only approximately 20% of the oocysts may sporulate (Graat *et al.*, 1994). Thus a change of 25% is, in this case, still inaccurate to cover possible

values as observed in practice or trials. So, the default value of α was changed more than the supposed variability of 25%. The level at which economic loss is minimal shifts from 68.2 to 78.2 oocysts, when the model was run with more realistic values of sporulation rate. This leads to a difference in net revenue of DFL 0.0056 (DFL 0.1706 − DFL 0.1650) (1 DFL $\approx$ 0.56 US$). So, an accurate estimation of this parameter does not lead to a substantial change of the outcomes, even with a change of 75% from the default.

Biological systems often are characterized by the fact that relatively few factors and their interactions exert a dominant influence on system performance (Benefield & Reed, 1985). This sensitivity analysis showed that the factors "immunity" and "anticoccidial efficacy" and their interaction are dominant on performance of the simulation model of the coccidiosis problem. In these situations, the behaviour of the system might be effectively controlled through control of these few factors (Benefield & Reed, 1985).

The reason for a drop in body weight gain (Figure 2), feed efficiency and consequently the net revenue at a 20% increased level of anticoccidial efficacy may be explained with the phenomenon of compensatory growth (Voeten, 1987). With a 15% increased level the threshold level, after which infection negatively effects production characteristics, is reached on day 16. At that time growth rate declines. Since 27 days are left in the flock cycle, there is enough time for compensatory growth to occur. At a 20% increased level, infection has no influence, so immunity is not developed. However, at day 35 the threshold level is reached and there is a great influence on growth rate. Since only 8 days until the end of the flock cycle are left, there is no opportunity for compensatory growth. With a 25% increased anticoccidial efficacy the threshold is not reached and values of production characteristics are almost equal to the values of non-infected flocks.

In conclusion, from this sensitivity analysis it appears that priorities should be placed on accurate estimation of the model parameters concerning anticoccidial efficacy and host immunity with respect to maximum attainable immunity, the rate at which maximum immunity is attained and time lag between parasite contact and start of building up immunity. Moreover, better understanding of immunity and mechanisms of immunity becomes more and more important, especially since resistance to anticoccidial drugs is a major restriction in the successful control of negative effects of coccidiosis (Chapman, 1993; Lillehoj & Trout, 1993).

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PART II

Experimental validation

Rate and course of sporulation of oocysts of *Eimeria acervulina* under different environmental conditions

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Rate and course of sporulation of oocysts of *Eimeria acervulina* under different environmental conditions

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ABSTRACT

An experiment was conducted to determine rate and maximum percentage of sporulation of *Eimeria acervulina* oocysts at various environmental conditions relating to temperature (21°C *vs.* 33°C) and relative humidity (RH) (40% *vs.* 80%). Measurements were made during 44 hours after excretion of oocysts in 3 substrates: dry litter, clammy litter and pure faeces respectively. Maximum sporulation percentage in both dry (22.6%) and clammy litter (19.5%) was higher ($P<0.005$) than in pure faeces (11.6%). Neither temperature nor RH had a significant influence on percentage of oocysts that sporulated. Under these simulated practical conditions approximately 25% of all oocysts sporulated, whereas sporulation under optimal conditions (29°C, aeration, 2% $K_2Cr_2O_7$) showed a higher (68%) sporulation ability of oocysts. At 33°C sporulation proceeded at a faster pace than at 21°C ($P<0.005$). With respect to RH and substrate, once sporulation started, rate of increase to maximum percentage was not different. Time of onset of sporulation was influenced by temperature ($P<0.0001$) and RH ($P<0.001$). Time of onset occurred 15 hours later at 21°C compared with 33°C and 5 hours later at 40% RH compared with 80%. Also, an interaction effect ($P<0.01$) was found with effect of RH being stronger at 21°C compared with 33°C. It was concluded that the most important aspect in the epidemiology of *E. acervulina* during a flock cycle is the time of onset of sporulation with temperature being the most important factor.

Key words: *Eimeria acervulina*, coccidiosis, sporulation, environmental conditions

INTRODUCTION

In the modern broiler industry great economic losses occur due to coccidiosis, which is caused by *Eimeria* spp. Until now, attempts to eradicate infections with *Eimeria* spp.

have failed and hence the negative effects of this parasitic infection on technical and econo-mic results persist. Control strategies mainly consist of continuous use of anticoccidials as food additives. Resistance against anticoccidials is an incentive to develop effective vaccines (Bafundo, 1989). An alternative approach is to model *Eimeria* infections in broilers. This might lead towards a better understanding of the complex problem, because many factors may be involved in the etiology of *Eimeria* infections (Henken *et al.*, 1992). The basic model describes the course of infection with *Eimeria* spp. in broilers. Infection occurs when broilers ingest sporulated oocysts. Infected broilers excrete oocysts with their faeces, of which a number will sporulate. Subsequently, a number of sporulated oocysts is ingested by the same or other chickens, hereby spreading the infection.

As only sporulated oocysts are infective, it is important to assess the rate of sporulation in time and the maximum number able to sporulate. Oocysts sporulate within 48 hours and *E. acervulina* oocysts even within 17 hours with often a maximum percentage between 85 and 95% if maintained under optimal conditions ($29 \pm 1°C$, O_2, 2% $K_2Cr_2O_7$) (Edgar, 1954, 1955; Reid, 1973; Lee & Shih, 1988). In poultry practice, conditions are suitable for the sporulation process regarding the occurrence of *Eimeria* infections. However, sporulation in practice has not been studied yet. The objective of the current study was to determine the rate and maximum percentage of sporulation under various conditions as they may occur in poultry practice. The experiment was conducted within the framework of building a simulation model for coccidiosis in broilers on technical and economic results.

MATERIAL AND METHODS

Sporulation assessment

In poultry practice, the temperature at the start of a flock cycle is kept at about 33°C and is declined until 21°C. The relative humidity is maintained between 40 and 80% (Weaver & Meijerhof, 1991). Therefore, sporulation was measured at 21 and 33°C temperature and 40 and 80% relative humidity (RH). Sporulation at combinations of temperature and relative humidity was assessed in 3 different substrates: 3 gram of wood shavings + 5 gram of faeces (referred to as S_1 = dry litter), 3 gram of wood shavings + 5 gram of faeces + 25 ml water (referred to as S_2 = clammy litter), and 5 gram of faeces (referred to as S_3 = pure faeces) respectively. The addition of water in S_2 was to imitate the litter condition around drinking places. To assess the effect of temperature, RH and type of substrate on sporulation, a split-plot design was used with substrates being nested within temperature and relative humidity. Measurements at each combination of temperature and

RH were repeated once (referred to as replicate 1 and replicate 2).

The substrates were kept in Petri culture-dishes in climatic-respiration chambers (Verstegen et al.,1987). Each gram of faeces contained $7.9 \times 10^5 \pm 4.1 \times 10^4$ (SEM) oocysts and had a dry matter content of 20.5%. The conditions chosen were representing the maximum contrasting environmental conditions in practice. Results, therefore, should reflect the maximum differences possible to exist.

On basis of two preliminary test results the following time-points for measurement of sporulation were determined: $t = 0$, 4, 6, 8, 10, 12, 14, 16, 18, 22, 32, 38, 44 hours after excretion of faeces. For each time point and substrate a duplicate measurement was made, resulting in 13×2 is 26 petri dishes for each substrate within a combination of temperature and relative humidity. In substrate 2 at $t = 8$, 22 and 32 respectively 5, 5, and 15 ml water was added to keep the litter clammy. As a control sporulation was also measured under optimal conditions ($29 \pm 1°C$, aeration, 2% potassium dichromate) (Reid, 1973). The oocysts were not separated from faecal material. Oocyst concentration in the suspension was 2.4×10^4/ml.

For practical reasons it was not possible to count all samples immediately. Therefore, in a pilot test it was determined whether sporulation stops at 4°C. According to Chakravarty & Kar (1946) sporulation ceases at this temperature. In the pilot test it appeared that sporulation percentage after 2 days at 4°C was not significantly different from the percentage assessed immediately. Therefore, sample suspensions were stored in the refrigerator at 4°C until analysed. Storage did not exceed two days.

To samples of substrates 1 and 2 tap water (50 ml) was added. Samples were then homogenized for 2 minutes and the mixture poured into a bowl through a sieve (mesh size ~ 1 mm) to remove large debris. Substrate 3 was suspended in 50 ml water. After stirring, the sporulation count was made on an aliquot of the suspension. Salt flotation was not employed to avoid possible bias due to differential flotation of sporulated and unsporulated oocysts. Oocysts were counted as being sporulated when they had 4 sporocysts which can be seen at $400 \times$ magnification. There were two observers for determining sporulation percentage. In a pilot test a kappa value for determining test agreement of 0.87 was found for these observers with a 95% confidence interval from 0.76 to 0.98 (Martin et al., 1987).

Oocysts

Oocysts of *E. acervulina* (field strain) were obtained from the Poultry Health Centre (Doorn, The Netherlands). This *Eimeria* species is predominantly detected in the Netherlands (Braunius, 1980). Methods for handling the parasites, such as counting of oocysts, sporulation and isolation were performed according to the methods described by Ryley *et al.* (1976). Fresh, unsporulated oocysts were obtained from experimentally infected

chicks by collecting faeces within 1 hour after defaecation 5 days post-infection. One-day-old male broiler chicks (Hybro®) were reared in a wired floor chick battery with a 23 h light and 1 h dark cycle. Temperature at the start was 30-33°C and declined with 3°C each week to a minimum of 21°C. Feed without anticoccidial drugs, and water were available *ad libitum* with the exception that the chickens were starved overnight prior to infection. The chicks were infected at 14 days of age and at an average body weight of 419±3 (SEM) g. The preparation of the inoculum was done 1 day prior to infection. The 2% potassium dichromat, in which oocysts were stored, was removed by means of centrifugation. Subsequently the oocysts were disinfected with a solution of sodiumhypochlorite (available chlorine = 6 g/100 ml) for obtaining oocysts free from contaminants. Chlorine was removed by means of centrifugation and afterwards the sediment was washed out 5 times with tap water. Then water was added so that the infection dose of oocysts was contained in one ml. The number of oocysts was counted with a haemocytometer (Fuchs-Rosenthal). Inoculation with 10^5 sporulated oocysts was done directly into the crop of each chick with a 1 ml syringe. According to Hein (1968) this causes a low to moderate infection in 2-week-old chickens.

Statistics

The following formula was used to determine the minimum number of oocysts to be differentiated: n = $(t \times SD/L)^2$ (Snedecor & Cochran, 1980) in which t is the value of the normal distribution at a certain confidence level(α), SD the standard deviation of the percentage of sporulation which is expected and L the maximum allowable error. With α=0.95, L=0.10, and expected sporulation proportion of 0.50 at least 96 oocysts have to be differentiated. With a proportion lower or higher than 0.50 fewer oocysts need to be differentiated, because SD is largest at 0.5. Viewing at least 96 oocysts is therefore the safest strategy.

Sporulation results were averaged over duplicates and observers. Curves were fitted for each combination of temperature, relative humidity and substrate per replicate. The percentage of oocysts that had sporulated by time t, was fitted by a logistic function of the form: y = $A/(1 + e^{-b(t-c)})$, using a non-linear least squares technique with the program NONLIN (Shareware program). The fitted curve is a 3 parameter model of the percentage of oocysts sporulated at time t. Parameter A represents the maximum sporulation percentage, b is the rate of increase in sporulation percentage at the inflexion point and c is time point of inflexion. The effect of temperature, relative humidity and substrate on these parameters was tested by analysis of variance. A model according to the split-plot design of the experiment was used (procedure GLM, SAS, 1989). The statistical model in which parameters were tested was:

$Y_{ijkl} = \mu + T_i + RH_j + (T \times RH)_{ij} + e_{ijk} + S_l + f_{ijkl}$,

where, Y_{ijk} — the value of A, or b or c, μ = overall mean, T_i = effect of temperature (i = 1,2), RH_j = effect of relative humidity (j = 1,2), $(T \times RH)_{ij}$ = effect of interaction between T and RH, e_{ijk} — error term 1, which represents the variation between replicates within temperature and relative humidity, S_l = effect of substrate (l = 1,2,3), f_{ijkl} — error term 2, which represents variation within substrates. The effect of temperature and relative humidity as well as the interaction between temperature and relative humidity were tested for significance against error term 1. The effect of substrate was tested against error term 2. The interactions between substrate and temperature, and substrate and relative humidity as well as the three-way interaction were not significant and consequently not included in the model. Results of the control, sporulation under optimal conditions, were not used in the statistical analysis, but served to evaluate the capacity of sporulation of the used oocysts.

Table 1. Estimated values of the parameters A, b, c for temperature, relative humidity and substrate per replicate when a logistic function was fitted to the percentage of oocysts sporulated with the percentage of variance explained (R^2) and the residual sum of squares (RSS).

Temp.	RH	S	Replicate	A	b	c	R^2	RSS
21	40	1	1	28.67	0.38	28.99	99.96	0.39
21	40	1	2	15.11	0.63	27.30	99.80	0.89
21	40	2	1	21.03	0.35	25.78	99.74	2.35
21	40	2	2	18.77	0.34	28.17	99.85	8.32
21	40	3	1	11.05	0.61	26.24	99.99	0.01
21	40	3	2	9.18	0.44	27.75	99.91	0.08
21	80	1	1	26.51	0.47	19.58	98.81	19.19
21	80	1	2	29.78	0.31	20.89	97.09	55.40
21	80	2	1	21.30	0.53	19.69	97.22	29.65
21	80	2	2	20.69	0.75	16.75	88.01	147.11
21	80	3	1	17.17	0.96	20.65	98.90	8.02
21	80	3	2	14.96	0.47	17.54	95.72	23.44
33	40	1	1	30.04	0.38	11.71	97.49	23.78
33	40	1	2	16.30	0.99	9.98	98.27	8.46
33	40	2	1	28.21	0.64	8.19	92.02	115.49
33	40	2	2	16.04	0.70	10.23	99.55	2.11
33	40	3	1	12.07	1.84	8.19	82.41	62.41
33	40	3	2	12.67	0.71	10.46	86.75	44.92
33	80	1	1	20.78	0.82	6.62	83.20	139.92
33	80	1	2	13.29	0.57	7.18	91.85	22.61
33	80	2	1	20.30	0.50	7.62	91.04	69.29
33	80	2	2	9.73	0.83	6.36	82.97	32.53
33	80	3	1	10.25	9.02	6.06	93.71	12.50
33	80	3	2	7.80	1.13	7.48	89.20	12.89
Optimal conditions				67.81	1.40	5.99	99.82	16.26

RESULTS

The estimated values of parameters are given in Table 1 with the percentage of variance explained by the model (R^2) and the residual sum of squares (RSS).

At 33°C temperature and 40% relative humidity samples from 32, 38 and 44 hours were dehydrated. These data were left out of the analysis. Also, from 8 other samples the faeces were dehydrated at sampling time 44 and consequently not used. The error terms of the statistical model were used to test normality. The value of 9.02 for rate of increase at 33°C, 80% RH, substrate 3, replicate 1 did not fit in the normal distribution and was therefore not used in further analysis. The maximum sporulation percentage under optimal conditions was 68%. Figure 1 shows the fitted logistic function.
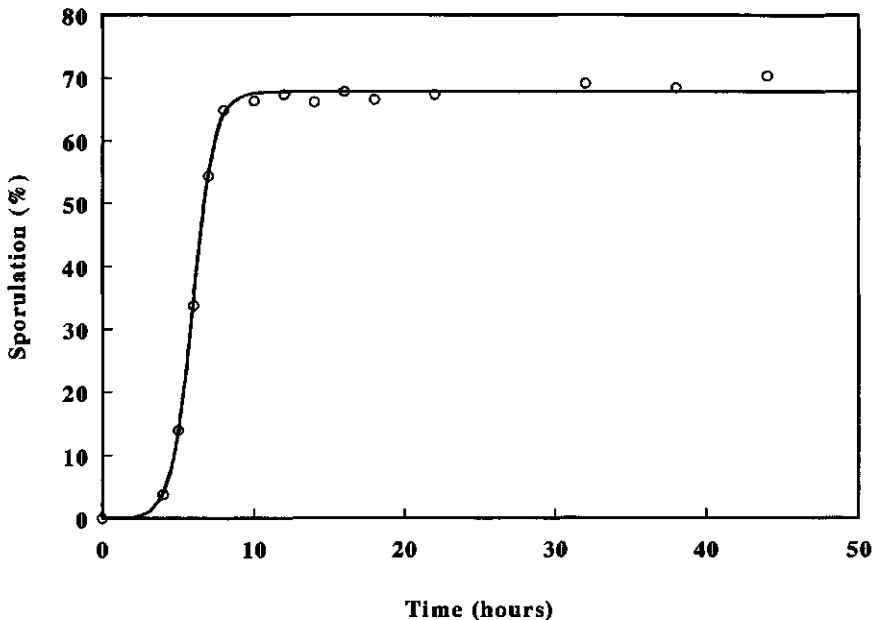
Figure 1. Fitted logistic curve for sporulation of oocysts of *Eimeria acervulina* under optimal conditions *i.e.* 29±1°C, aeration, 2% potassium dichromate.

An effect of temperature or relative humidity on maximum sporulation percentage (A) was not found. At both temperatures approximately 18% of the oocysts sporulated. The same percentage was found at both relative humidities. Between substrates, however, a significant ($P < 0.0001$) difference in maximum sporulation percentage existed. Sporulation percentage in substrate 3 (11.9%) remained significantly ($P < 0.005$) lower compared with substrate 1 (22.6%) and 2 (19.5%). Figures 2 & 3 show the estimated sporulation curves.

Figure 2. The effect of temperature on the percentage of *Eimeria acervulina* oocysts which sporulate in three different substrates. o − − −o: 21°C in S_1, □− − −□: 21°C in S_2, ∇− − − ∇: 21°C in S_3, ●———●: 33°C in S_1, ■———■: 33°C in S_2, ▼———▼: 33°C in S_3. The curves consist of least square means of the parameters of the fitted logistic function.

For the rate of increase (b), only temperature was found to have a significant effect ($P < 0.005$). The least square means of b at 21°C and 33°C were 0.52 and 0.86 respectively. For relative humidity and substrate it is shown that once sporulation had started the rate of increase to maximum sporulation was not significantly different (Figures 2 & 3).

The time of inflexion (c) was significantly influenced by temperature ($P < 0.0001$) and by relative humidity ($P < 0.001$), but not in a uniform way. This is shown by the interaction effect between temperature and relative humidity ($P < 0.01$). No significant effect of substrate on c was found. The mean of point of inflexion at 21°C was at 23.3 hours and for 33°C at 8.4 hours. Figure 2 shows this difference. The difference between 40 and 80% relative humidity was smaller, 18.6 compared with 13.1 hours (Figure 3). The interaction effect is demonstrated in Figure 4. The figure shows that the effect of relative humidity on the point of inflexion is stronger at 21°C compared with 33°C.
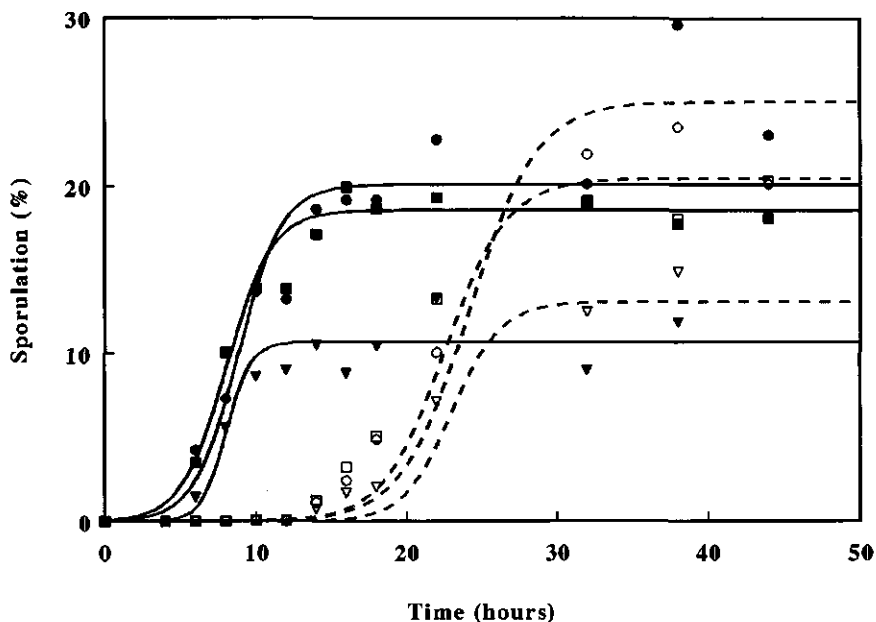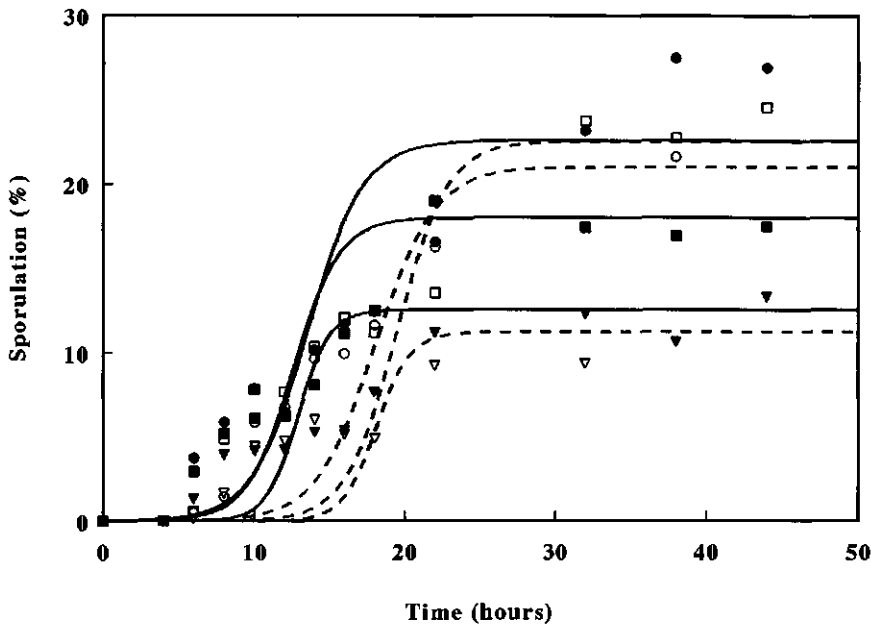
Figure 3. The effect of relative humidity on the percentage of *Eimeria acervulina* oocysts which sporulate in three different substrates. o– – –o: 40% in S_1, □– – –□: 40% in S_2, ▽– – – ▽: 40% in S_3, ●———●: 80% in S_1, ■———■: 80% in S_2, ▼———▼: 80% in S_3. The curves consist of least square means of the parameters of the fitted logistic function.

DISCUSSION

The sporulation rate of oocysts under environmental conditions, as occurring in poultry practice, has not been studied so far. However, in a flock cycle, the number of sporulated oocysts and course of sporulation is an important factor in terms of the level of challenge to which broilers are exposed, thus influencing the epidemiology of *Eimeria* spp.

The present experiment was conducted within the framework of building a simulation model for effects of *Eimeria* infections in broilers on technical and economic results. It was designed to determine sporulation under maximum contrasting circumstances encountered in practice. In a model of coccidial infections (Parry *et al.*, 1992) a sporulation percentage of 70% was used, which was based on sporulation in floor-pen trials. According to these researchers the degree of sporulation might depend on such conditions as humidity and temperature. The maximum percentage of oocysts of *E. acervulina* that sporulated did not differ between temperatures or relative humidities investigated in the present study. Parry *et al.* (1992) used a time delay parameter for sporulation of oocysts, which was based

on known sporulation rates under laboratory conditions. However, they mentioned a possible influence of temperature and humidity. In the present experiment these factors influenced both the time of onset of sporulation and the velocity at which sporulation occurred. Onset of sporulation occurred later for oocysts incubated at 21°C or at 40% relative humidity. The interaction between temperature and relative humidity may be interpreted as a larger impact of relative humidity at lower temperatures, with a relative humidity of 80% being more favourable for sporulation.
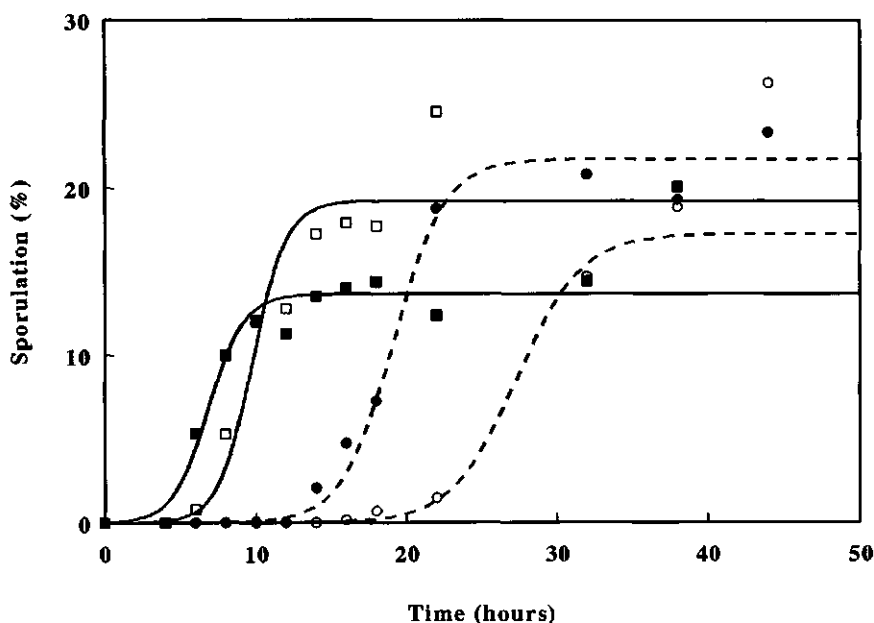
Figure 4. The percentage of *Eimeria acervulina* oocysts sporulated when incubated at 21°C and 40% RH(o – – –o), 21°C and 80% RH(● – – – ●), 33°C and 40% RH(□————□), 33°C and 80% RH (■————■). The curves consist of least square means of the parameters of the fitted logistic function.

In faeces on dry litter or clammy litter more oocysts sporulated compared with faeces alone. Faeces *per se* may be a poor medium because of a rapid loss of moisture resulting in an anaerobic environment. There was no difference in maximum sporulation rate between dry litter and clammy litter. So, from this point of view it might be concluded that broilers at places around drinkers do not have a higher risk for getting infected with *Eimeria*, unless a larger number of oocysts is excreted around drinkers. However, Long & Rowell (1975) examined variations in numbers of oocysts per gram of surface litter according to sampling position. Possible differences due to drinkers were not found.

Practical conditions which are favourable for growth of broilers are suitable for the sporulation process regarding the problems in eradicating *Eimeria* infections. The results of this experiment are in agreement with that fact. The infectivity of the apparently completed sporulated oocysts is not taken into account. This is another aspect which requires further research. In this experiment, it appeared that at conditions simulating those occurring in practice approximately only 25% of all oocysts sporulate whereas the control indicated a high ability of sporulation (about 68%). The difference between the sporulation percentage of 85 to 95% under optimal conditions as reported by several research workers (Edgar, 1954, 1955; Lee & Shih, 1988) and the percentage under optimal conditions in this experiment is probably due to the fact that our control oocysts were not separated from faecal debris. Microbial growth could have been responsible for the poorer sporulation, despite the addition of 2% potassium dichromat and also because the larger amount of material requiring aeration (Ryley *et al.*, 1976).

Since oxygen is required for sporulation (Reid, 1973), it is presumed that there was not enough aeration in the Petri dishes as it was in the control which had forced aeration all the time. Supposedly, if oocysts are under actual practical conditions the percentage of sporulation is higher as a consequence of the activity of the broilers thereby aerating the litter. Horton-Smith & Long (1954) also mentioned the disturbance of the litter being favourable for oocysts. Nevertheless, the sporulation rate is still not that high as under optimal conditions, and thus a very important factor in the dispersion of the parasite. With a very large number of oocysts present in the broiler house, the impact of a lower sporulation percentage will be relatively smaller, but still there will be an influence on spreading of the parasite. Anticoccidials are also important in the epidemiology of the parasite, primarily in reducing oocyst output. Yet, many anticoccidials used in poultry practice have also proved to give a reduced sporulation rate (Joyner & Norton, 1977; Von Löwenstein & Kutzer, 1989). Ruff *et al.* (1978) found besides a decreased sporulation rate, also a lower infectivity of the sporulated oocysts. From an epidemiological view the sporulation percentage is not the most important factor, since the results are almost equal for all conditions, as studied in this experiment. The time of onset of sporulation is possibly of more influence with regard to the timing of infection of broilers during a flock cycle. The possibility of compensatory growth is dependent on the timing of infection and with that the economic revenue. It was concluded that the most important aspect in the epidemiology of *E. acervulina* during a flock cycle is the time of onset of sporulation with temperature being the most important factor.

ACKNOWLEDGEMENTS

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Effects of initial litter contamination level with *Eimeria acervulina* on population dynamics and production characteristics in broilers

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Effects of initial litter contamination level with *Eimeria acervulina* on population dynamics and production characteristics in broilers

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ABSTRACT

The present experiment was done to obtain experimental evidence supporting the existence of an optimal initial contamination level of *Eimeria acervulina* with respect to broiler productivity, as suggested by simulation model outcomes. Three levels of initial (on Day 3) contamination of the litter were applied (low, intermediate and high). The peak of oocyst excretion during the flock cycle (grow-out) (Days 0-36) depended on the initial contamination level. Oocysts peaked at Day 15, 22, and 33 for the high, intermediate, and low contamination level, respectively. Antibody titres and plasma carotenoid contents were not significantly affected by contamination levels. Average body weight at the end of the flock cycle (Day 36) and average daily body weight gain were significantly higher at the intermediate contamination level compared with the low and high contamination levels. Average body weight at Day 36 was 1681 g, 1712 g and 1674 g for the low, intermediate, and high contamination level, respectively. Average daily weight gain was 45.7 g, 46.5 g and 45.5 for the low, intermediate, and high contamination level, respectively. Thus, the data from this experiment support the existence of an optimum initial contamination level for *E. acervulina* with respect to performance results.

Key words: chicken, *Eimeria acervulina*, oocyst shedding, growth performance

INTRODUCTION

Infections caused by *Eimeria* spp. continue to cause significant economic losses in the broiler industry (Lillehoj & Trout, 1993). Control measures include the use of anticoccidial drugs in the feed and the application of hygienic measures. Anticoccidial drugs generally have a limited use since parasites can become resistant to these (Chapman, 1993). From this point there is a considerable interest in developing vaccines to prevent the

negative effects of coccidiosis. However, owing to the complex life-cycle of the parasite and incomplete understanding of its interaction with the host, development of an efficient vaccine is slow (Lillehoj & Trout, 1993). Alternatively, there may be other ways to control *Eimeria* spp. infections.

Henken *et al.* (1994a,b) developed a computer model simulating the population dynamics of an *Eimeria acervulina* infection in broilers and its possible effects on various production traits. This model may be useful in giving an insight into the epidemiology of *E. acervulina* infection and effects on broiler productivity and may provide the means for developing and testing new strategies to combat coccidiosis. One of the most interesting computer simulation results was the finding that productivity and net return depended on the initial contamination of oocysts in the litter in a parabolic way. An intermediate initial contamination level resulted in a higher average daily gain, a better feed to gain ratio, and a higher net revenue compared with high and low initial contamination levels. This suggests the existence of an optimum initial contamination level. The present trial was conducted to obtain experimental evidence for such an optimum initial contamination level. It was demonstrated that antibody titre and carotenoid content in plasma were useful criteria for evaluation of intestinal coccidial infection (Saatara Oz *et al.*, 1984; Yvoré *et al.*, 1993). Therefore, an additional objective was to estimate antibody titres and carotenoid levels as potential parameters of monitoring infection levels for this type of experiment in which infection takes place continuously.

MATERIAL AND METHODS

Experimental chickens and housing

One-day-old broiler chickens (Hybro®; Euribrid, Boxmeer, Netherlands; n = 1704) were reared in floor pens (n = 12) with wood shavings (3 kg/m²) up to 36 days of age. The pens had solid walls, 80 cm high, and were 6 m² (2.40×2.50 m) in size. With 142 chickens assigned at random to each pen, chick density was 23.7/m². Ambient temperature at the start was set at 33°C and was decreased by 3°C/wk to a minimum of 21°C. The lighting regimen was a 23/1 h light/dark cycle. Standard commercial feed without anticoccidial drugs and water were available for *ad libitum* consumption and were supplied to each pen separately. The feed given the first 21 days was a commercial starter ration (crude protein CP) 22.33%, metabolisable energy (ME) 2973 kcal/kg) and from Day 21 onwards, a commercial grower ration (CP 21.54%, ME 3024 kcal/kg), both containing 30 mg/kg carotenoids. The high carotenoid content was chosen to enable the measurement of any change in plasma carotenoid content after infection with *E. acervulina* (Ruff & Fuller, 1975).

Coccidial isolate and oocyst dose

Oocysts of *E. acervulina* (field strain) were obtained from the Poultry Health Centre(Doorn, Netherlands). Three initial contamination levels were used, related to the outcomes of the simulation model as presented by Henken *et al.* (1994b) (Table 1) together with a control group (no contamination). The number of oocysts per contamination level was higher in the present experiment than indicated in the model. This was because the computer model assumes that all oocysts in a house are available for ingestion by the broilers. In practice, many oocysts get lost in the litter and will therefore be not available to the broilers. Infection of the litter was done at Day 3 of the experiment (Day 0 is start of flock cycle or grow-out).

Table 1. Initial contamination levels (oocysts per broiler) in simulation model (Henken *et al.*, 1994b) and present experiment.

Infection level	Simulation model	Present experiment
Control	0	0
Low	0.17	1
Intermediate	17	100
High	1700	10000

Each pen was divided into nine square sections. The oocyst dose was divided into nine portions. Using a pipette, one portion (sporulated oocysts in 1 ml tap water) was placed on top of the litter in the middle of each section on Day 3 of the experiment. Control pens received the same treatment, except that 1 ml tap water only was used for each section.

Experimental design

The chick house was divided into three blocks of four pens. Within a block, contamination levels were randomly assigned to pens. Each contamination level comprised three replicate pens of 142 birds per pen.

Measurements

Production characteristics. Body weight was recorded twice a week by weighing six groups of ten randomly chosen birds per pen. Feed intake and water consumption were also recorded twice a week (total of a 3 and a 4 day period).

Levels of carotenoids and antibody titres in plasma. Blood samples were drawn by wing vein puncture twice a week starting on Day 8. Plasma was isolated from the blood samples

through centrifugation. Each plasma sample was divided into two portions and stored at −20°C before analysis.

Carotenoid analysis was done using a modification of the method described by Allen (1987). Briefly, 100 μl of plasma was added to 900 μl of acetone to precipitate the protein, followed by centrifugation for 10 min at 1500×g. Supernatant (250 μl) was put on microtitre plates and optical densities were determined at 450 nm.

Antibodies to *E. acervulina* were quantified by ELISA using a modification of the methods used by Galmes *et al.* (1991). Soluble antigens were prepared from suspensions of *E. acervulina* obtained from the Poultry Health Centre, according to the method of Rose (1977). The antigen, antiserum and conjugate dilutions were optimised before use. The soluble antigen was diluted in carbonate buffer pH 9.6 with a final protein content of 50 μg/ml. All incubations were for 1 h at 37°C and the plates were washed three times with water containing 0.05% Tween®20 (Merck-Schuchardt, Hohenbrunn, Germany) in between each step except when reported otherwise. The reaction volumes were 100 μl unless stated otherwise.

Plates, coated with antigen, were incubated, washed and stored at −20°C until use. Phosphate buffered saline (PBS) pH 7.2 containing 0.2% bovine serum albumin (BSA; Sigma Chemical Co., Bornem, Belgium) and 0.05% Tween®20 was used as diluent. Plasma, diluted in four steps with a starting dilution of 1:20, was added to the plates and incubated. All plates (polystyrene microtitre plates; Greiner, Alphen a/d Rijn, Netherlands) contained a duplicate positive reference plasma. Subsequently, 100 μl conjugate (rabbit anti chicken IgG (H+L)/PO; Nordic Immunological Laboratories, Tilburg, Netherlands) used at a dilution of 1:10000 were added and incubated. A solution of tetramethylbenzidine (TMB; Sigma) was used as substrate and allowed to incubate for 10 min at room temperature in the dark. The reaction was stopped with 50 μL 2.5 N H_2SO_4. Absorbance values were read at 450 nm with a multichannel spectrophotometer (Titertek Multiskan; ICN Biomedicals, Zoetermeer, Netherlands).

Other measurements. The oocyst content of litter samples (a mixed sample of nine handfuls taken in nine sections per pen) was measured twice a week. Counting of oocysts was done according to Long & Rowell (1975). Other records included daily mortality observations.

Statistical analysis

On the 19th Day of the flock cycle, oocysts were observed in the litter of the control pens. Therefore, data of these controls were left out of the analysis.

Each response variable was examined as a function of the design and treatment structure using the General Linear Models (GLM) procedure of the Statistical Analysis Systems Institute Inc. (SAS, 1989). The model was:

$$Y_{ijk} = \mu + A_i + B_j + e_{ijk},$$

where, Y_{ijk} is the kth observation in the ith block for the jth treatment, μ is the overall mean, A_i is a fixed effect of the ith block ($i=1,2,3$), B_j is a fixed effect of the jth treatment ($j=1,2,3$), and e_{ijk} is the error term. The values presented in text and tables are least square means with their standard error (SEM), corrected for the factors in the model used. Mortality data were used to perform survival analysis using the LIFETEST procedure of SAS (1989).

RESULTS

Figure 1 shows the temporal pattern in the number of *E. acervulina* oocysts detected in the litter for each contamination level. Each curve consists of least squares means and their SEM as generated by statistical analysis. Oocyst excretion reached a peak on Day 15, 22, and 33 for the high, intermediate and low level, respectively. Differences between contamination levels were found at Day 12 ($P<0.001$), 15 ($P<0.05$), 19 ($P<0.01$) and 22 ($P<0.10$).

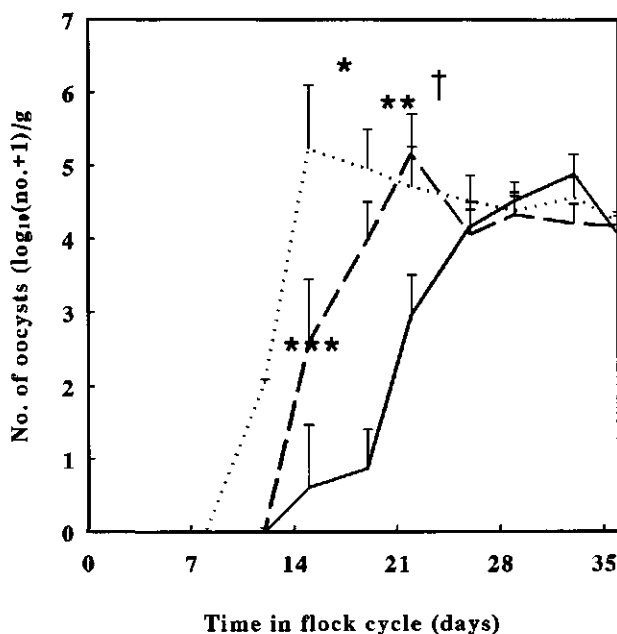**Figure 1.** Number of *Eimeria acervulina* oocysts per gram (^{10}log(no.+1)) of litter as a function of time (days in flock cycle) for the low (———), intermediate (– – –) and high (·····) initial contamination level. Values represent the least square means + SEM (SEM vary from 0.0 to 0.86; n=3). $^{\dagger}P<0.10$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Table 2. Body weight (BW) at Day 36, body weight gain (BWG) from Days 0 to 36, cumulative feed to gain ratio (FGR), and cumulative water to feed intake ratio per initial contamination level in experiment and simulation model.

Infection level	Experiment				Simulation model[a]		
	BW (g)	BWG (g/d)	FGR (g/g)	Water:feed (g/g)	BW (g)	BWG (g/d)	FGR (g/g)
Low	1681[b]	45.7[b]	1.548	1.85	1611	43.6	1.59
Intermediate	1712[a]	46.5[a]	1.586	1.84	1701	46.2	1.55
High	1674[b]	45.5[b]	1.585	1.87	1653	44.8	1.56
SEM	8	0.3	0.012	0.01	----------------- b -------------------		

[a]Adapted to present production level.
[b]SEM can not be calculated from deterministic models.
Means within a column followed by different letters differ significantly (BW, $P<0.05$; BWG, $P<0.10$).

The body weight of broilers at the high initial contamination level was lower than at the intermediate and low levels at Day 22 ($P<0.10$; SEM = 11), Day 26 ($P<0.05$; SEM = 9) and Day 29 ($P<0.05$; SEM = 5). At Day 36, body weight was higher ($P<0.05$; SEM = 8) for the intermediate initial contamination level (1712 g) than for the low (1681 g) and high (1674 g) initial contamination levels (Table 2). Results remained similar when corrected for body weight at the start.

Body weight gain became reduced for the first time around Day 15, 19, and 22 at the high, intermediate, and low level, respectively.

Feed to gain ratio differed between initial contamination levels at Day 12 ($P<0.05$), 19 ($P<0.10$), 22 ($P<0.05$), 26 ($P<0.01$), 29 ($P<0.01$), and 33 ($P<0.01$) (Figure 2). At Day 36, no statistical significant differences between contamination levels in this ratio were observed (Table 2).

Mean (±SEM) cumulative mortality was 4.9±1.2% and survival rate did not differ between infection levels.

The mean (±SEM) water to feed intake ratio declined from 2.30±0.04 at the start of the experiment to 1.85±0.01 at the end, and was not different between initial contamination levels (Table 2).

Table 2 also shows body weight at Day 36 and average daily gain from Day 0 to 36 as generated by the computer simulation model.

From Day 8 to 12, the mean (±SEM) antibody titre declined from 1.3±0.16 to 0.3±0.11). From Day 12 onwards, antibody titre increased to between 3.1 and 3.6±0.15 on Day 36. Except for Day 19, significant differences between contamination levels were present (P ranging from 0.0001 to 0.0730; SEM ranging from 0.08 to 0.25); however, differences between contamination levels were not consistent throughout the trial period.
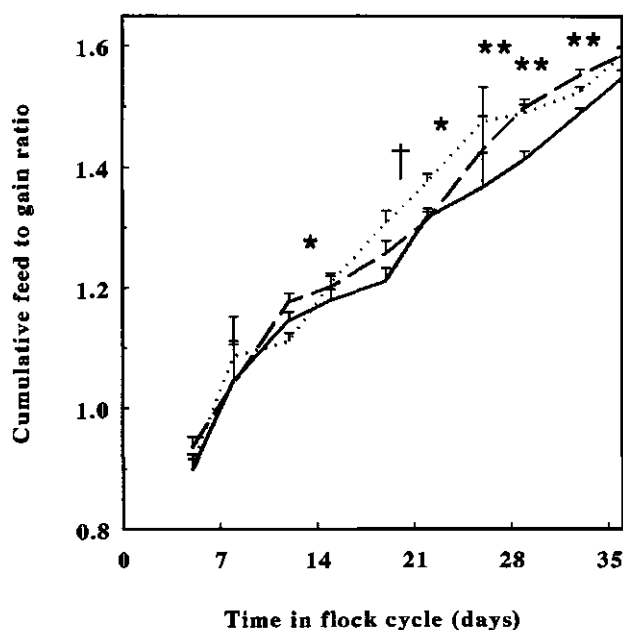
Figure 2. Cumulative feed to gain ratio during the flock cycle (days) for the low (———), intermediate (– – –), and high (······) initial contamination level of *Eimeria acervulina*. Values represent the least square means + SEM (SEM vary from 0.008 to 0.065; n=3). $^{\dagger}P<0.10$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Figure 3 shows the carotenoid content in plasma. On Day 15, carotenoid content differed between groups ($P<0.05$), being highest for the low initial contamination level and lowest for the high initial contamination level. On Days 19 and 22, carotenoid content was lower ($P<0.05$) for the high initial contamination level than for the low and intermediate levels. On Day 26 the low contamination level showed a higher ($P<0.05$) carotenoid content compared with the intermediate and high levels, whereas on Day 33 the carotenoid content was lower ($P<0.10$). On Day 36, the low initial contamination level still showed the lowest carotenoid content, although the differences with the other groups were not significant.

DISCUSSION

The present experiment was done to obtain experimental evidence supporting the existence of an optimal initial contamination level of *Eimeria acervulina* with respect to

broiler productivity, as suggested by computer simulation model outcomes (Figure 4) (Henken *et al.*, 1994a,b).

In this experiment, oocysts peaked at Day 15, 22 and 33 for the high, intermediate and low contamination level, respectively. Respective computer simulation outcomes indicated peak numbers on Day 13, 21, and 27. In an experiment by Voeten & Braunius (1981) with an initial contamination level similar to the intermediate level used in this experiment, oocysts of *E. acervulina* reached a peak at Day 21, which is close to Day 22 in our experiment. Average body weight and body weight gain at the end of this experiment was highest for the intermediate initial contamination level, the same result as found with the computer model, although differences between contamination levels were smaller in this experiment than in the computer model (Table 2). In this experiment body weight gain became reduced for the first time around Day 15, 19 and 22 for the high, intermediate, and low initial contamination level, respectively, *vs.* Day 16, 18 and 24 in the computer simulation model.

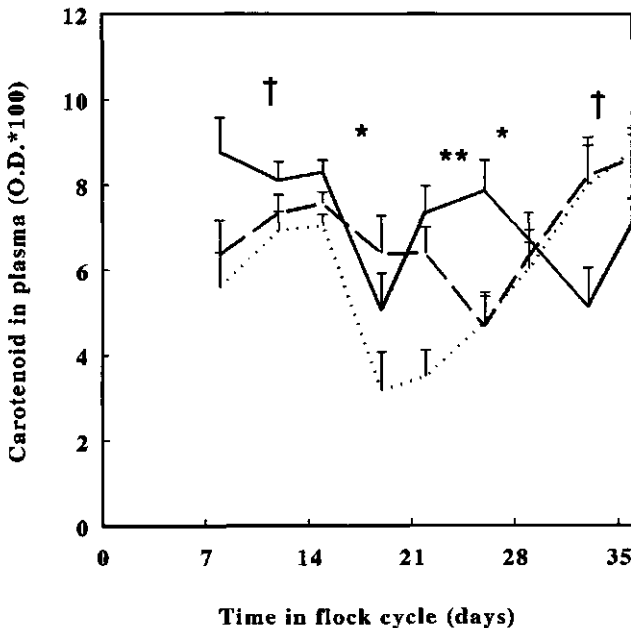**Figure 3.** Carotenoid content in plasma (optical density $\times 10^2$) of broilers during the flock cycle (days) after contamination of the litter at Day 3 for the low (————), intermediate (– – –) and high (·····) initial contamination level of *Eimeria acervulina.* Values represent the least square means + SEM (SEM mean vary from 0.21 to 0.94; n=3). $^{\dagger}P<0.10$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.
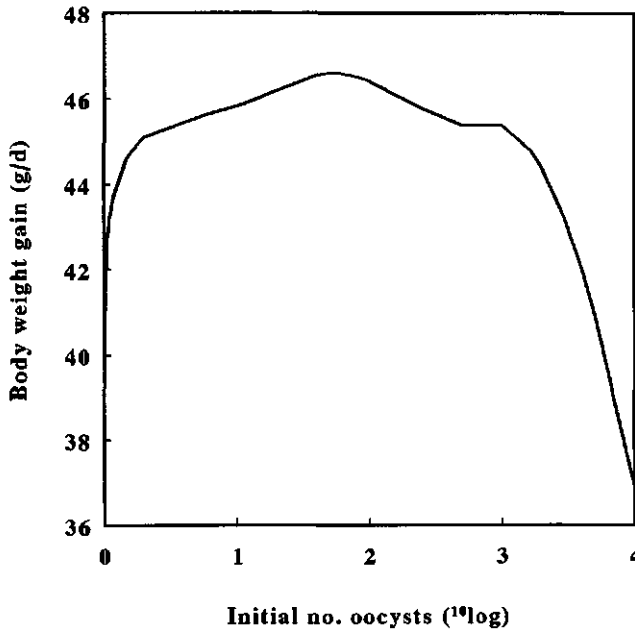
Figure 4. Body weight gain (g/d) per broiler during a flock cycle in relation to initial contamination level as found in the computer simulation model (see also Henken *et al.*, 1994b).

These results show that from a qualitative perspective the model behaves as in reality, *i.e.* this experiment, and that it might be expected that there is an optimum infection level at which economic loss per broiler is lowest. Thus, the results support the hypothesis generated by the simulation model (Henken *et al.*, 1994b). However, quantitative differences demonstrate that agreement between the computer simulation model and experiment was less satisfactory.

The quantitative differences might be caused by the fact that control pens became infected. Therefore, it is likely that contrasts between other contamination levels were reduced as well, as a result of mutual contamination between pens. However, this does not change the conclusions concerning the qualitative behaviour of the model. The effects found might have been larger if cross-contamination had not occurred, thus more strongly supporting adequate behaviour of the computer model.

Antibody titres and plasma carotenoid levels were determined because the literature indicated that these could be used to adequately reflect differences in the course of infection depending on initial contamination levels. Rose (1978) stated that the presence of circulating antibodies is an indication of developing or recently acquired immunity. Saatara Oz *et al.*

(1984) showed that antibody response is directly related to the number of oocysts inoculated in 2- to 16-week-old chickens. The absorption of carotenoids from the diet is affected by coccidiosis (Yvoré, 1978). Ruff *et al.* (1974) indicated a decrease in plasma pigmentation with *E. acervulina,* which occurred as early as 4 days post infection and persisted for up to 14 days. Furthermore, they found that the magnitude of the decrease in plasma pigmentation is dependent on the number of oocysts ingested by the bird.

In the present experiment differences in antibody titres between groups were not significant or not consistent between initial contamination levels and no dose-response relationship was found. Thus, a relation with number of oocysts ingested and developing immunity and antibody titre was not found in the current experiment. This might be explained with interference of maternal immunity, age effect and timing of infection. Smith *et al.* (1994) demonstrated that *Eimeria maxima* IgG antibodies were transferred via the egg yolk to hatchlings. So, the antibodies, which were detected between Day 8 and 12 might have interfered with antibody production. By the time maternal antibodies disappeared, many oocysts were present in all groups resulting in similar increases in antibody titre. Also, in this experiment carotenoid contents could not be used to consistently discriminate between the three initial contamination level groups. Yvoré *et al.* (1993) stated that measurement of serum coloration is a very good criterion for evaluating intestinal coccidial infection, and that even small numbers of oocysts lead to a significant decrease in serum coloration. This was not very clear in our experiment, but plasma decoloration already may have occurred before the start of sampling on Day 8.

Another reason for the lack of clear contrasts in antibody titre and carotenoid contents in the plasma between the different initial contamination levels might be the mutual contamination between pens.

In conclusion, given the experimental results, validation of the model (Henken *et al.*, 1994a,b) showed a qualitative agreement. However, this does not imply validity of the model under different circumstances, nor does it imply validity of each part of the model (Shannon, 1975; Martin *et al.*, 1987; Black *et al.*, 1993). Nevertheless, the results of present experiment provided more confidence in the model as a whole.

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Eimeria acervulina: influence of corticosterone-induced immuno-suppression on oocyst shedding and production characteristics in broilers, and correlation with a computer simulation model

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Eimeria acervulina: influence of corticosterone-induced immunosuppression on oocyst shedding and production characteristics in broilers, and correlation with a computer simulation model

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ABSTRACT

An experiment was conducted to investigate the effects of immune responsiveness on excretion of oocysts after *E. acervulina* infection and subsequent effects on production characteristics of broilers (*Gallus domesticus*). These effects were determined in broilers repeatedly infected with 2.85×10^3 oocysts of *E. acervulina* and treated with various dosages of corticosterone in the diet (0, 10, 20 and 30 ppm). Corticosterone treatment did not have an effect on the peak oocyst excretion, although it was administered from 4 days before initial infection. The number of oocysts excreted shortly after the peak and the length of the excretion period were increased in corticosterone treated groups. The absence of a difference in peak oocyst excretion was ascribed to the existence of a time-lag between first contact with the parasite and coming into operation of protective immunity. In a recently developed computer simulation model this period was assumed to be 5 days. Assuming that immunosuppression, through corticosterone, only is effective when protective immunity is in operation, the results indicate a time-lag of at least a few days, which supports the inclusion of such a time-lag in the computer simulation model. General immunosuppressive effects of the corticosterone treatment, monitored by antibodies and mitogen induced lymphocyte stimulation confirmed that immunosuppression occurred shortly after medication started. Infection did not have a significant influence on production characteristics in animals without dietary corticosterone. However, with increasing corticosterone levels the negative effects of infection on production also increased.

Key words: *Eimeria acervulina*, chicken-protozoa, coccidiosis, immunity-protozoa, production systems

INTRODUCTION

Henken *et al.* (1994a,b) developed a simulation model to improve understanding of development and effects of coccidiosis in broilers. With this simulation model it is possible to focus on potentially relevant factors that affect the population dynamics, *i.e.* epidemiology, of an *Eimeria acervulina* infection and subsequently the productivity and thus final economic results. The model includes simulating the development of protective immunity against *E. acervulina* infection. This immunity is the resultant of several aspects which are level of innate immunity, time-lag before immunity can develop after first contact with the parasite, maximum attainable level of protective immunity and the rate at which maximum attainable level of immunity is reached. For each component as well as for the resultant immunity, parameters can be used to study the relative impact on model output, which is called sensitivity analysis. Sensitivity analysis showed that even slight changes in the values of these parameters had enormous effects on the model output body weight gain, feed to gain ratio and net revenue per broiler (Graat *et al.*, submitted). This indicates that immune responsiveness in general as well as each of its separate components may be of major importance in the epidemiology of coccidiosis. Testing validity of the model is important and has to be done by comparing predictions with (experimental) results (Black *et al.*, 1993). Consequently, it was decided to study these aspects experimentally to obtain data either verifying or falsifying the computer simulation results.

Chicken immune responses to *Eimeria spp.* are complex and involve both humoral and cellular immune mechanisms. Cell-mediated immune responses are generally considered to be most important in the defense against coccidial infections. Attempts to suppress immune responsiveness were often done to gain information on the mechanism involved in resistance to reinfection with *Eimeria* (Rose, 1970; Lillehoj & Trout, 1994). Some immunosuppressive agents have a general effect on humoral immune response, others on cell-mediated responses, and again others on both. Corticosteroids have general suppressive effects on immune response by influencing the production and functioning of lymphocytes. Therefore and also since *E. necatrix* infections in corticosteroid treated birds were more severe than in untreated chickens (Gross *et al.*, 1980), corticosterone was used as the immunosuppressive agent.

The objective of this study was to determine the effect of various dosages of corticosterone on oocyst output and production traits in broilers infected with *E. acervulina*. The initial goal was to suppress immunity development in general.

MATERIAL AND METHODS

Chickens

At Day 1 of the experiment, 144 one-day-old male chickens of a commercial strain (Hybro[*]; vaccinated for infectious bronchitis and Newcastle Disease at day of hatching).

Husbandry

Chickens were kept in wired floor cages (n = 24) until the end of the experiment at 42 days of age with 6 birds per cage (0.6 × 0.75 m). Cages were placed in each of two identical climatic-respiration chambers, chambers 1 and 2 (Verstegen *et al.*, 1987). Chicks were randomly assigned to the cages. Temperature at the start was set at 33°C and was automatically declined to a minimum of 21°C at Day 26, which was maintained till Day 43. Relative humidity was maintained at 60%. Until Day 5 lights were on continuously. From Day 5 onwards the lighting schedule was a 23-h light and 1-h dark cycle. Standard commercial feed without anticoccidial drugs, and water were available *ad libitum*. From Day 7 onwards feed with corticosterone was given (based on Gross *et al.*, 1980).

Treatments

The study was conducted as a 2 × 4 factorial arrangement of treatments with two levels of infection (infection (chamber 1) *vs.* no infection (chamber 2)) and 4 levels of corticosterone (ICN Biomedical Inc., Ohio) in the feed (0, 10, 20 and 30 ppm), resulting in 8 treatment groups. Each treatment group consisted of 3 observations, *i.e.* 3 cages with 6 chicks per cage. Each chick in the infection group received 2.85×10^3 sporulated oocysts of *Eimeria acervulina* (strain from Houghton Poultry Institute (U.K.), maintained and kindly provided by Janssen Pharmaceutica N.V. (Belgium)), contained in one ml tap water, on Days 11, 14, 18, 21, 25, 28, 32, 35, and 39 directly in the crop with a 1-ml syringe. The number of oocysts in the inoculum was determined with a haemocytometer (Fuchs-Rosenthal). This infection dose corresponds to infection doses as used in the experiment of Stiff & Bafundo (1993) and to the simulation model (Henken *et al.*, 1994a,b), giving clear development of protective immunity. The infection dose was meant for induction of a subclinical infection as it was in the computer simulation model. Each chick in the non-infection group was given 1 ml of tap water directly into the crop. Corticosterone was used to suppress general immunity. It should be noted, however, that corticosterone affects a wide range of activities in the body; it has a profound effect on metabolism of carbohydrates, proteins, and fat (Hsu & Crump, 1989).

Measurements

Birds were weighed individually at Days 0, 7, 11, 14, 18, 21, 25, 28, 32, 35, 39, and 42. Feed intake was measured per cage for the 11 periods determined by the above mentioned weighing days. Feed intake was adjusted for mortality of chicks. Mortality was determined daily. Dead birds were weighed and sent to the Poultry Health Centre to determine cause of death.

On Days 7, 11 and from Day 14 onwards every other day, a faeces sample was collected per cage, mixed, and examined in duplo for oocysts using a McMaster counting chamber according to the method of Long & Rowell (1958). All faeces excreted between sampling days was determined by starting each period with a weighed clean empty faeces tray beneath each cage and weighing it at the end of the period.

Blood samples were drawn by wing vein puncture twice a week from day of infection, *i.e.* Day 11, until the end of the 6-week experiment. Plasma was isolated from the blood samples through centrifugation. Each plasma sample was divided in two portions and stored at -20°C until analysis. Plasma carotenoid level was determined as a potential parameter for monitoring course of infection, as it is known that even very low levels of infection are reflected in a lowered content of carotenoid in the plasma (Conway *et al.*, 1993). Carotenoid analysis was done according to Allen (1987). Similarly, plasma antibody titres were assessed as potential parameter to monitor infection and to check whether or not corticosterone had an immunosuppressive effect. Antibodies were quantified by the enzyme-linked immunosorbent assay (ELISA) according to Galmes *et al.* (1991), [For a description of the methods see also Graat *et al.* (1996)].

On the first day of infection (Day 11) and one week after initial infection (Day 18), eight animals in each corticosterone group were bled for Concanavaline A (ConA) lymphocyte stimulation tests (LST). This was done to verify for corticosterone induced suppression of T-cell responsiveness. Peripheral blood leucocytes (PBL) were obtained from 1 ml of sterile heparinized blood using Ficoll density gradient centrifugation. In brief, 1 ml blood was layered on 0.7 ml Ficoll-Paque (Pharmacia). This was centrifuged for 1.5 min at 11,500 rpm in an Eppendorf centrifuge, and then lymphocytes were collected and counted. Lymphocytes were washed twice with RPMI-1640 (Roswell Park Memorial Institute) (centrifugation for 10 minutes at 400 g) and suspended in RPMI to give a concentration of 5×10^6 PBL/ml. Each well of sterile 96-well flat-bottomed plates was filled with 100 μl of the cell suspension + 100 μl of ConA (2 μg/ml RPMI-1640), set up in triplicate. This was incubated for 48 hours at 41°C and 5% CO_2. After 30 hours, 0.4 μCi methyl-^{3}H-thymidine in 20 μl was added. Tritium-thymidine uptake was determined with a Beckman ß-scintillation counter. Results were expressed as mean stimulation indices (SI). The SI were calculated as counts per minute in ConA-stimulated cultures divided by counts per minute

in unstimulated cultures.

At Day 43 chickens were killed by intravenous administration of T61 (Hoechst Holland N.V.). T61 is a fluid containing a strong narcoticum (200 mg/ml embutramide), a muscle relaxant (50 mg/ml mebezoniumjodide), and an anaestheticum (5 mg/ml tetracainehydrochloride), and is meant for euthanasia without an excitation phase (0.3 ml/kg body weight). Each chicken was checked on gender and abnormalities and the bursa of Fabricius and spleen were removed and weighed.

Statistical analysis

Differences in treatments were determined by subjecting data to analysis of variance. Analyses of results were done on cage averages for each parameter, since cage is the experimental unit and not the individual animal. To analyse data of body weight, body weight gain, feed conversion ratio, oocyst excretion, carotenoid content of plasma, antibody titre, Stimulation Indices of LST (SI) and relative weight of bursa and spleen, the following statistical model was used (PROC GLM, SAS, 1989):

$$Y_{ijk} = \mu + A_i + C_j + (A \times C)_{ij} + e_{ijk},$$

where, μ = overall mean, A_i = effect of infection, $(i = 1,2)$, C_j = effect of corticosterone level $(j = 1,2,3,4)$, $(A \times C)_{ij}$ = interaction effect between infection and corticosterone, e_{ijk} = error term, which represents variation between cages within treatment groups. Data on mortality were subjected to proportional hazard regression (PROC PHREG, SAS, 1989).

Table 1. Corticosterone intake (mg/kg body weight) (mean ± sd) per infection level and treatment group.

Treatment group	Uninfected	Infected
10	24.7[a] ± 1.2	23.9[a] ± 1.3
20	62.3[b] ± 0.8	58.9[b] ± 1.0
30	100.1[c] ± 7.3	99.8[c] ± 3.8

[a,b,c]Means within rows and columns lacking a common superscript differ significantly (P = 0.0001)
Note. Corticosterone intake was calculated from feed intake from Day 7 to Day 42 and body weight at Day 42.

RESULTS

Corticosterone intake per treatment group related to body weight was significantly different ($P = 0.0001$) (Table 1), showing that the aimed difference in corticosterone dose was realised. Stimulation of lymphocytes with ConA showed a dose-response relationship with corticosterone 4 days after administration began, *i.e.* on Day 11. Stimulation indices of the

4 corticosterone treatments on Day 11 were negatively correlated with increasing corticosterone levels. However, this effect was not significant ($P=0.1702$). Indices had a value of 127, 95, 81 and 22 (SEM=35; n=6 per corticosterone level) for corticosterone 0, 10, 20 and 30 ppm, respectively. For the ConA test lymphocytes were used in a standard concentration of 5×10^6/ml. The number of cells per ml blood as sampled at each test day, however, showed a significant negative correlation with the amount of corticosterone administered (Day 11: $r=-0.52$, $P=0.0037$; Day 18: $r=-0.55$, $P=0.0011$). Activity of corticosterone was also reflected in antibody titres during the experiment (Figure 1) and weight of the organs bursa and spleen relative to body weight (Table 2).
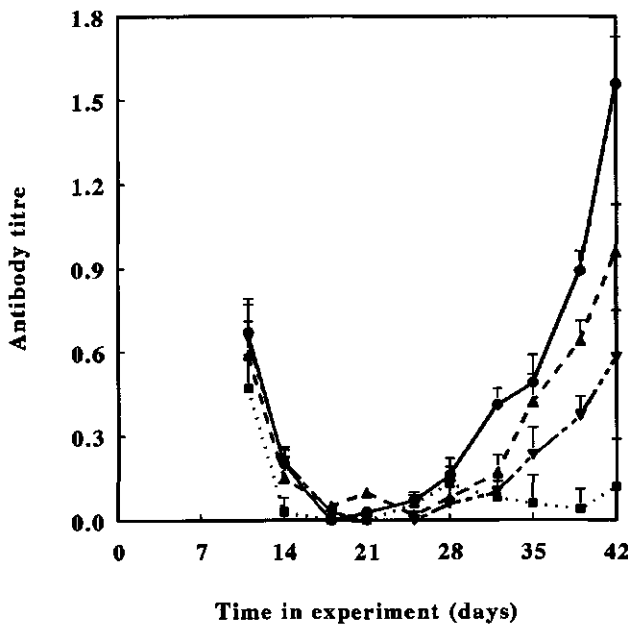
Figure 1. Antibody titre during the experiment for each corticosterone group (+SEM; n=3) (●———●: 0 ppm, ▲ – – – ▲: 10 ppm, ▼———– – ▼: 20 ppm, ■······■: 30 ppm).

Figure 1 shows the antibody titres for *E. acervulina* during the experimental period for each corticosterone level. From Day 32 until the end of the experiment antibody titres differed significantly between corticosterone groups (from $P=0.0156$ to $P=0.0001$).

The relative weight of both bursa and spleen was significantly influenced by corticosterone level ($P=0.0001$) and were negatively correlated with the dose ($P=0.0001$ for both correlations). Relative weight of both bursa and spleen was smaller in infected animals than in uninfected animals, although the difference was not significant (Table 2).

Table 2. Relative weight of bursa and spleen (as percentage of body weight multiplied by 10^2) on Day 43 with their standard error of mean and correlation coefficient with corticosterone dose.

Corticosterone	Bursa	Spleen
0	16.9[a]	12.6[a]
10	8.3[b]	8.6[b]
20	4.9[c]	7.6[b]
30	4.2[c]	4.8[c]
SEM	1.0	0.7
r	-0.86	-0.84
Uninfected	8.8	8.8
Infected	8.3	8.0
SEM	0.7	0.5

[a,b,c]Means within a column lacking a common superscript differ significantly (P=0.0001)
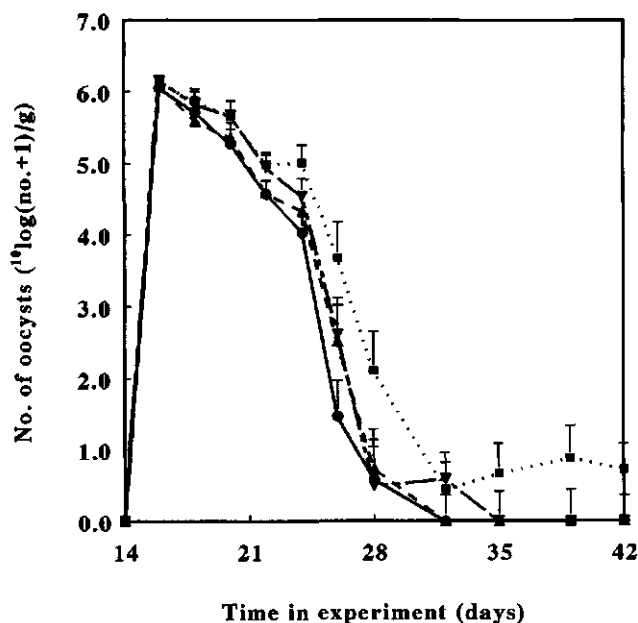
Figure 2. Oocyst production (+SEM; n=3) per corticosterone group (0, 10, 20 and 30 ppm) after repeated infection with 2.85×10^3 *Eimeria acervulina* starting on Day 11 (●———●: 0 ppm, ▲ – – – ▲: 10 ppm, ▼———– – ▼: 20 ppm,■·······■: 30 ppm).

Figure 2 shows the oocyst excretion during the experiment. At Day 16 the peak of excretion is reached, after which excretion declines. No difference was found in the peak of oocyst excretion between treatment groups. After this peak, numbers of oocysts in the different corticosterone groups started to deviate from each other. However, only at Day

26 the difference between corticosterone levels tended to be significant (*P*=0.08). At the corticosterone levels 0, 10 and 20 ppm no oocysts were found any more from Day 32, 32 and 35 onwards, respectively. At 30 ppm corticosterone oocysts could be detected throughout the experiment.
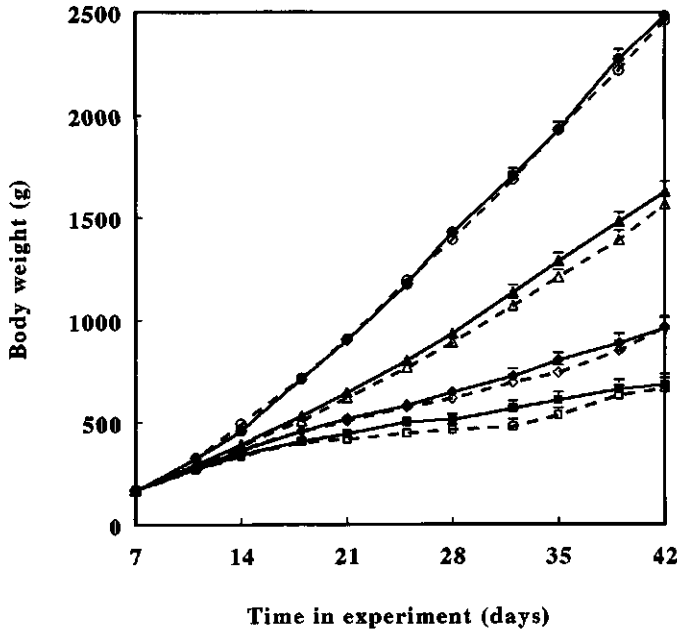
Figure 3. Body weight (+SEM; n=3) for infected (open symbols, dotted lines) and uninfected (closed symbols, straight lines) animals in each corticosterone group. Corticosterone medication started at Day 7.

Figure 3 shows body weight development during the experiment. From 4 days after start of corticosterone administration (Day 11) towards Day 42 body weight was significantly affected by corticosterone level (*P* < 0.0001). Without corticosterone no effect of infection on body weight could be seen. With 10, 20 and 30 ppm corticosterone, body weight of the infected animals was lower (*P* < 0.10) than that of the uninfected animals until Day 35. Infection appeared to reduce body weight to a greater extent at higher corticosterone levels (Figure 4).

At 10, 20 and 30 ppm corticosterone, average daily gain from Day 11, *i.e.* day of initial infection, was lower in the infected than in the uninfected animals until the end of the experiment, although only significantly (*P* < 0.05) until Day 21, 10 days after initial infection.

Figure 5 shows the feed conversion for infected and uninfected animals as averaged over corticosterone groups. Differences in feed conversions caused by infection were not

obvious, although feed conversion seemed higher the first 10 days after infection, in the infected group. Feed conversion was significantly ($P < 0.0001$) increased with increasing corticosterone dose.
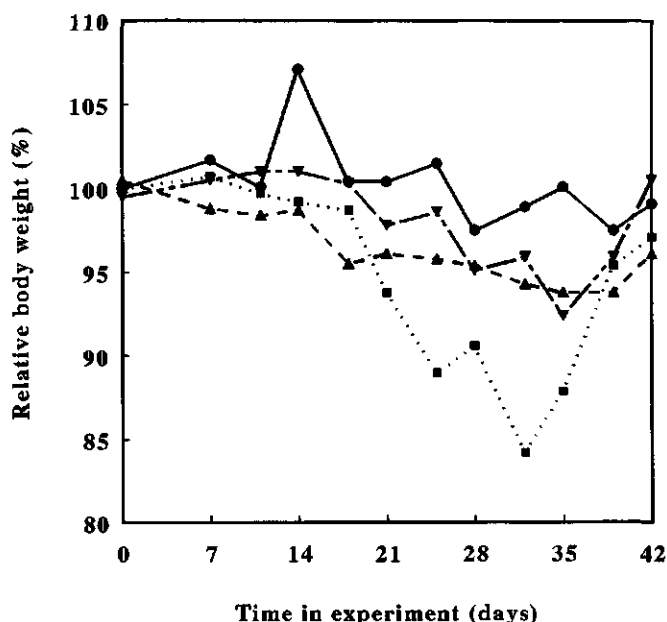
Figure 4. Relative body weight of infected animals compared to uninfected animals within each corticosterone group (●———●: 0 ppm, ▲– – –▲: 10 ppm, ▼———–▼: 20 ppm, ■······■: 30 ppm).

Figure 6 shows the carotenoid levels in plasma for infected and uninfected chickens. After first infection on Day 11, carotenoid in plasma of infected chickens at Day 18 was significantly lower ($P < 0.0001$) than in uninfected chickens. Until Day 28 differences remained significant ($P < 0.05$). Towards the end of the experiment carotenoid level in the infected animals returned to the level as found in the uninfected animals. At all measuring days, except at Day 42, a significant effect of corticosterone on carotenoid level was present ($P < 0.005$) resulting in higher carotenoid levels at higher corticosterone levels, due to the increased feed conversion. There was no interaction effect between corticosterone treatments and infection treatments.

At day of infection (Day 11), the lymphocyte stimulation index was similar for infected and uninfected chickens, being respectively 80 and 82 (SEM = 25; n = 16). However, one week after infection the Con-A induced proliferation of peripheral blood lymphocytes (PBL) was significantly lower ($P = 0.0159$) for infected (SI = 17) than uninfected (SI = 61) chickens.
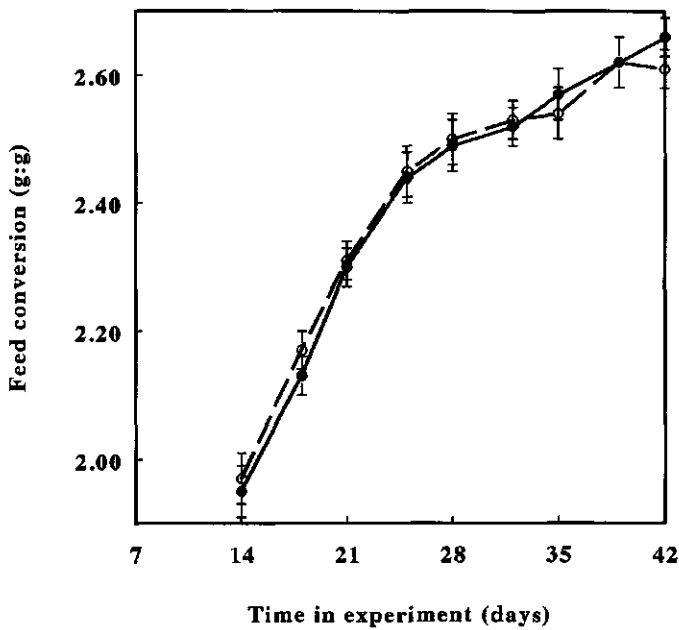
Figure 5. Feed conversion (+SEM; n = 3) calculated from Day 11 for infected (o – – –o) and uninfected (•———•) chickens (averaged over corticosterone groups).

Figure 6. Carotenoid in plasma (extinction × 10³ + SEM; n = 3) for uninfected (•———•) and on Day 11 initially infected (o – – –o) broilers (averaged over corticosterone groups).

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Mortality during the experimental period was 20% on average (n = 29). No apparent effect of infection with *E. acervulina* on risk for dying was found (relative hazard 1.40; *P* = 0.45). Use of corticosterone, however, in a dose of 20 or 30 ppm was associated with an increase in the risk for death (relative hazard = 4.19 and 5.56, respectively; *P* = 0.07 and *P* = 0.03 respectively).

D I S C U S S I O N

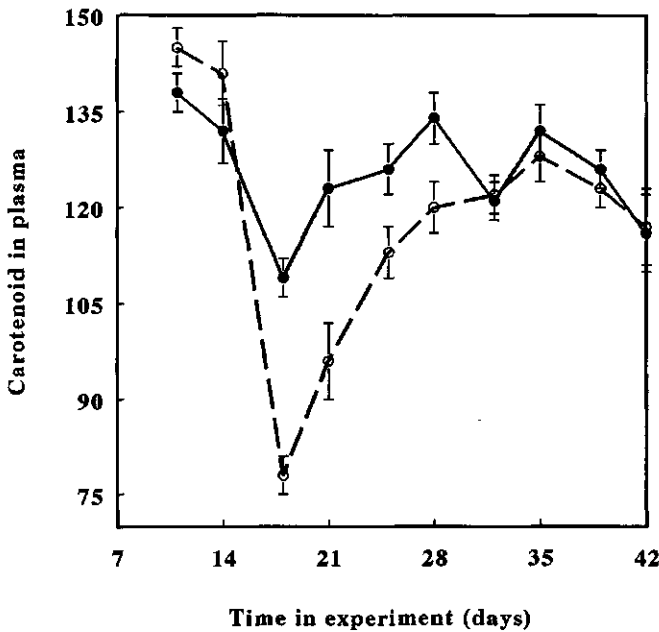In the present experiment broiler immune responsiveness was suppressed using different dosages of corticosterone to test the hypothesis that with a lowered general immune responsiveness the effects of an *E. acervulina* infection on broiler production are much larger. This hypothesis was derived from sensitivity analysis of a computer simulation model for *Eimeria acervulina* infection in broilers (Henken *et al.*, 1994a,b; Graat *et al.*, submitted). Numerous studies have been conducted in which the immune response has been suppressed. Most of these studies aimed at elucidating immune response mechanisms using oocyst shedding as the main criterion instead of production characteristics, and focused on other species than *E. acervulina* (Long & Rose, 1970; Rose, 1970; Lillehoj, 1987; Rose *et al.*, 1989; Isobe & Lillehoj, 1993). In the present study, effects of production characteristics were also investigated.

Effects of corticosterone
The lymphocyte stimulation test, ELISA for measuring antibodies, and weighing the organs bursa and spleen were done to investigate whether corticosterone treatment had an immunosuppressive effect. All tests indicate that corticosterone treatments did suppress immune responsiveness, and that the extent of suppression increased with increasing corticosterone level, as has been observed by others (Gross *et al.*, 1980; Davison & Flack, 1981; Lillehoj, 1986; Isobe & Lillehoj, 1992; Isobe & Lillehoj, 1993; Martin *et al.*, 1994).

Effects on oocyst excretion
Effects on course of oocyst excretion. The influence of corticosterone level did not have an effect on peak oocyst excretion. However, the magnitude of excretion shortly after the peak and the length of the excretion period was increased in corticosterone treated groups. Several studies showed a prolonged patency period of *Eimeria mivati* after administration of an immunosuppressor compared to control chicks (Long & Rose, 1970; Rose, 1970; Isobe & Lillehoj, 1993). Results of our study show a longer excretion period after repeated infection in corticosterone treated birds, however, differences were less marked than in

above mentioned studies. Probably, this is due to the fact that in the present study the immunosuppressor was administered orally, instead of intramuscularly. Isobe & Lillehoj (1993) found intramuscular administration to be more effective than oral administration. Davison *et al.* (1983) described 3 disadvantages of the dietary route of administration. First, intake of corticosterone depends on daily feeding pattern, but since broilers eat constantly small portions during the lighting period (Gordon, 1995) this appears to be of minor relevance. Secondly , digestive breakdown occurs and thus the effective dose is difficult to assess. However, half-life of intramuscularly injected corticosterone is short, requiring regular injections. The latter also requires often handling of the animals which may be considered as a major disadvantage. Thirdly, variation in uptake of individual animals within treatment groups may occur. Nonetheless, administration via the diet results in a more constant uptake of corticosterone than through injections, and was therefore chosen in the experiment. The longer excretion period in immunosuppressed animals might be ascribed to a somewhat slower rate of attainment of maximum immunity.

Effects on peak oocyst excretion. In experiments of Rose (1970), and Isobe & Lillehoj (1993) as well as in our study no actual difference was found in peak oocyst excretion between untreated groups and groups treated with an immunosuppressor. Long & Rose (1970) even found a lower peak in immunosuppressed animals and ascribed this to extended schizogony. Furthermore, they explained the higher oocyst production later as a result of slower, and because of this, more efficient multiplication. The similar oocyst peak excretion in corticosteroid treated and untreated chicks might also be explained with the existence of a time-lag between first contact with the parasite and developing immunity becoming effective. In the computer simulation model (Henken *et al.*, 1994a,b) it was assumed that this time-lag is 120 h (5 days). If an effective immunity already builds up during the first days after infection, it might have been expected that, due to immunosuppression, which started 4 days before initial infection, peak oocyst excretion would have differed between corticosterone treated and untreated infected groups. However, this was not the case, supporting the inclusion of such a time-lag in the computer model. This, however, assumes that corticosterone treatment shortly after application results in immunosuppression. Reduced lymphoproliferation at day of infection indicate that this may be the case. Nevertheless, further research is needed to confirm the hypothesis of existence of time-lag between first parasite contact and coming into effect of protective immunity.

Effects on production characteristics

The influence of immunity status, which was varied by corticosterone application, on body weight gain and feed conversion ratio after repeated infection with *E. acervulina* appears not as remarkable as in the computer simulation model. However, it is difficult to

draw an absolute comparison between the computer model and the experiment since the percentage of immunosuppression in the experiment is difficult to relate to the percentage in the computer model. Nevertheless, to study the phenomenon of a possible increased effect of infection due to immunosuppression, qualitative comparison is allowed. In the computer simulation model, body weight of infected animals relative to body weight of uninfected animals drops to 72 to 88% depending on level of general immune responsiveness. In our experiment, body weight of infected animals was 84 to 100%, depending on corticosterone level. Furthermore, in the computer simulation model infection with oocysts always leads to a lower body weight compared with uninfected animals even with a "normal", *i.e.* the default input value for immunity, or higher general immune responsiveness level (Figure 7).
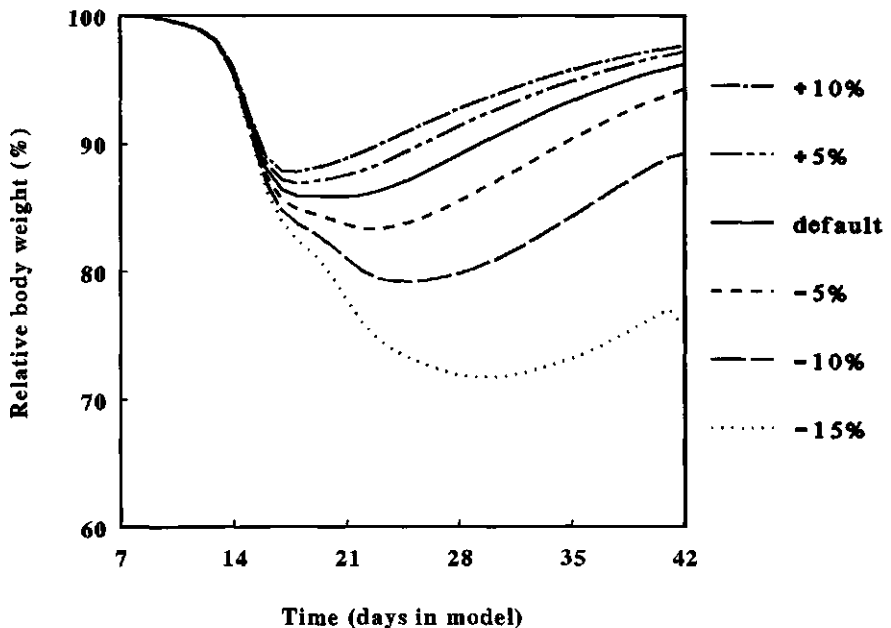
Figure 7. Body weight of infected chicks with different levels of general immune responsiveness relative to the body weight of uninfected controls as a result of computer simulation.

In our experiment growth and final body weight after repeated infection with *E. acervulina* in non-immunosuppressed animals was not lowered, so low levels of infection do not have a significant influence on production characteristics. With immunosuppression due to corticosterone treatment an effect of infection could be seen, in which increasing dosages corticosterone led to increasing effects (Figure 3). Overall, the results show that in a qualitative way the simulation model is in agreement with the results of the experiment.

However, in the model the effect of differences in general immune responsiveness appear to overestimate the effects as were observed in the experiment.

ACKNOWLEDGEMENTS

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Eimeria acervulina infection in broilers: aspects of build up protective immunity

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Eimeria acervulina infection in broilers: aspects of build up of protective immunity

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ABSTRACT

An experiment was conducted to verify whether corticosterone affects oocyst excretion immediately after application. As a result of previous experiment it was suggested that there is a time-lag between first contact with *E. acervulina* and coming into effect of protective immunity. In this previous experiment, immediate immunosuppression due to corticosterone application was shown by a decreased mitogenic lymphocyte stimulation on the day of infection. However, an immediate effect of corticosterone application on immunity against infection with *E. acervulina* in terms of reduced oocyst excretion had to be verified. The results of the present experiment clearly indicate that administering corticosterone immediately effects oocyst excretion, supporting the hypothesis that a time-lag exists between first contact with *E. acervulina* and build up of protective immunity, as proposed by a previous experiment. Results of present experiment combined with previous results, suggest that corticosterone treatment suppresses the effector function of protective immunity against *E. acervulina*, but does not prevent development of protective immunity itself.

Furthermore, results suggest that the time-lag between first infection and coming into effect of protective immunity depends to some extent on infection level. This was indicated by differences in oocyst excretion after repeated infection with 2.8×10^4 oocysts of *E. acervulina* compared with a ten times lower infection dose. Immunity in the lower dose (2.8×10^3) was reached with smaller negative effects on growth and feed conversion than in the higher dose (2.8×10^4).

Key words: *Eimeria acervulina*, coccidiosis, protective immunity, time-lag, infective dose

INTRODUCTION

Eimeria acervulina infects the intestinal epithelial cells of chickens, causing economic losses to poultry producers. Alternative strategies to control the disease coccidiosis are necessary as a result of increasing problems with drug resistance (Chapman, 1993).

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Furthermore, development of a vaccine is slow (Lillehoj & Trout, 1993) and not yet cost effective in broiler production. A computer simulation model of *E. acervulina* infection and its effects on broilers is being developed to gain better understanding of the interaction between host and parasite (Henken *et al.*, 1994a,b). Sensitivity analysis of this simulation model showed a substantial influence of immunity status on production characteristics in chickens infected with *E. acervulina* (Graat *et al.*, submitted[a]).

In a validation experiment for this simulation model (Graat *et al.*, submitted[b]), broilers had been given the immunosuppressive agent corticosterone (CO) by the feed. Four days after feeding CO commenced, broilers were repeatedly infected with *E. acervulina*. The immunosuppressed animals showed a longer oocyst excretion period compared with untreated animals. Immunosuppression had no effect on the level of peak oocyst excretion, which was found previously (Long & Rose, 1970; Rose, 1970; Isobe & Lillehoj, 1993). Because CO administration commenced four days prior to initial infection, this lack of effect of CO on peak oocyst production might be explained with a time-lag existing between first contact with the parasite and coming into effect of protective immunity. In the computer simulation model (Henken *et al.*, 1994a,b) it was assumed that this period is 5 days. The validity of a time-lag as suggested by the previous experiment critically depends on whether or not CO treatment results in suppression of protective immunity against *E. acervulina* immediately after its application at a time such immunity can be expected to have developed. In the present experiment this was investigated.

Additionally, in the computer simulation model the time-lag is a time delay between appearance of a parasite stage and its effect on the rate with which specific immunity is acquired. The maximal rate in this model depends on the cumulative amount of internal host parasite stages, which is the summation of cumulative numbers of new trophozoites, schizonts and oocysts. This means that the infection dose is important as far as build up of protective immunity is concerned.

In this experiment corticosterone administration started at 2 different times during the period of oocyst excretion to see whether application has an immediate effect on oocyst excretion. Furthermore, the influence of the magnitude of infection dose on establishing protective immunity was studied.

MATERIAL AND METHODS

Chickens

One hundred and forty four one-day-old male chickens (vaccinated for IB and NCD) of a commercial strain (Hybro®) were used for the six week experiment.

Husbandry

Chickens were kept in wired floor cages (n=24) until 39 days of age with 6 birds per cage (0.6×0.75 m). Cages were placed in each of two identical climatic-respiration chambers (Verstegen *et al.*, 1987). Chicks were assigned to 5 weight categories (<41, average of 42, 44 (2 groups), 46 and >48 g). Each of these 6 groups consisted of 24 chicks. From each weight category one chick was randomly assigned to a cage. Temperature at the start was set at 33°C and declined to a minimum of 21°C at Day 26, which was continued till Day 39. Relative humidity was maintained at 60%. Until Day 5 there was continuous light. From Day 5 onwards the lighting regime was a 23-h light and 1-h dark cycle. Standard commercial starter ration without anticoccidial drugs was given until Day 16. From Day 16 onwards the chickens were fed a commercial grower ration without anticoccidial drugs. Throughout the experiment, feed and water were available *ad libitum*.

Treatments

Infection dose with *E. acervulina* (strain from Houghton Poultry Institute maintained and kindly provided by Janssen Pharmaceutica N.V.) was the same as in a previous experiment (2.8×10^3) (Graat *et al.*, submitted[b]) and also a treatment with a 10 times higher dose was added (2.8×10^4). The study was conducted as a 3×3 factorial arrangement of treatments with three levels of infection (2.8×10^3 or 2.8×10^4 oocysts or no infection) and 3 corticosterone treatments (ICN Biomedical Inc.) (no corticosterone, or 20 ppm from Day 16 or from Day 25), resulting in 9 treatment groups. Corticosterone was administered in the feed. Treatment groups with no infection consisted of 2 observations, *i.e.* 2 cages with 6 chicks per cage, the other treatment groups consisted of 3 observations. Corticosterone dose was based on a previous experiment (Graat *et al.*, submitted[b]) and research of Gross *et al.* (1980). Chickens in the infection groups received the infection dose contained in one ml tap water, on Days 11, 14, 18, 21, 25, 28, 32, and 35 directly in the crop with a 1-ml syringe. The number of oocysts in the inoculum was determined with a haemocytometer (Fuchs-Rosenthal). Each chick in the no infection group was given 1 ml of tap water directly into the crop.

Measurements

Birds were weighed individually at Days 0, 7, 11, 14, 18, 21, 25, 28, 32, 35, and 39. Feed intake was measured per cage for the 10 periods determined by the above mentioned weighing days. The period between Day 14 and 18 was split in two periods since on Day 16 feed was changed to feed with corticosterone. Feed intake was adjusted for mortality of chicks. Mortality was determined daily. Dead birds were weighed and sent to the Poultry Health Centre to determine cause of death.

On Days 7, 11, 14, 16, 18, 20, 22, 24, 26, 27, 33, 35, 37, 39 a mixed sample of faeces produced during preceding days was collected per cage and examined in duplo for oocysts using a McMaster counting chamber according to Long & Rowell (1958). All faeces produced in the period between measuring days was measured by placing a weighed clean empty faeces reservoir beneath each cage and weighing it at the end of the period.

At Day 40 chickens were killed by intravenous administration of T61 (Hoechst). Each chicken was checked on gender and abnormalities, and the bursa of Fabricius, spleen and liver were removed and weighed.

Statistical analysis

Differences in treatments were determined by subjecting data to analysis of variance. The statistical model used (SAS, 1989) for oocyst excretion, body weight, relative body weight gain, feed to gain ratio, and relative weight of bursa, spleen and liver, was:

$$Y_{ijk} = \mu + A_i + C_j + (A \times C)_{ij} + e_{ijk},$$

where, μ = overall mean, A_i = effect of infection ($i=1,2,3$), C_j = effect of time of corticosterone administration ($j=1,2,3$), $(A \times C)_{ij}$ = interaction effect between infection and time of corticosterone administration, e_{ijk} = error term. Error terms were tested for a normal distribution. Data were considered normal when skewness and kurtosis were within -2 and +2 standard deviations.

Relative body weight gain was used since body weight at start of initial infection at Day 11 already differed significantly between treatment groups. Relative body weight gain per animal was calculated as growth in each period (between measuring days), divided by body weight at the beginning of that period multiplied by 100.

RESULTS

Figure 1a shows the pattern of oocyst excretion for each corticosterone treatment group for the infection with 2.8×10^4 oocysts (= "high") of *E. acervulina*. Peak of oocyst excretion was reached on Day 18 and was higher for the groups treated with corticosterone from Day 16 ($P < 0.10$). Excretion of oocysts in the groups receiving corticosterone from Day 25, immediately started to differ from the excretion in groups receiving no corticosterone ($P < 0.005$).

The excretion of oocysts after repeated infection with 2.8×10^3 oocysts (= "low") was not different between corticosterone treated and untreated groups until Day 22 (Figure 1b). After that, corticosterone treatment resulted in a higher excretion compared with untreated groups ($P < 0.05$). Again, corticosterone treatment at Day 25 immediately resulted in a

higher oocyst excretion in comparison with groups with no corticosterone (NS). The effect of corticosterone on excretion of oocysts resulted in significantly higher excretion compared to untreated groups (P varying from < 0.001 to < 0.05). At Days 16, 18, 20 and 22 the effect of infection resulted in significantly ($P < 0.0001$) higher excretion for the groups with the "high" infection level, whereas from Day 26 until Day 33 excretion was higher ($P < 0.05$) for the "low" infection level. Effects of corticosterone were highest in the "high" infected animals, which was reflected in a significant interaction effect ($P < 0.05$) on Days 20 and 22.

1 a. "high" infection: 2.8×10^4
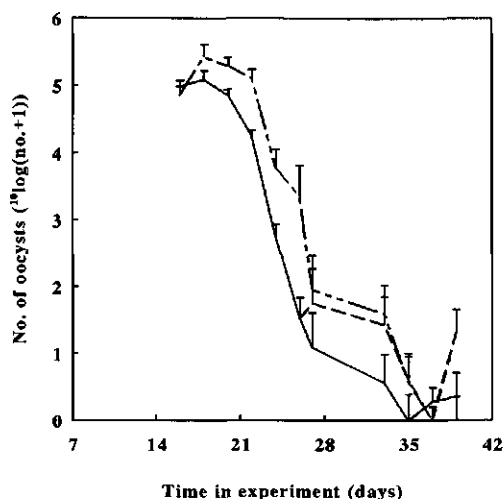
1 b. "low" infection: 2.8×10^3
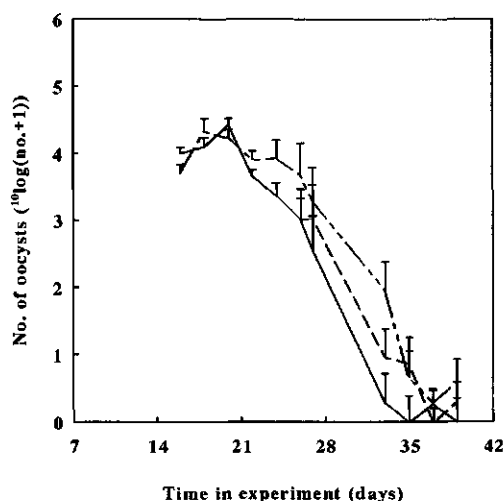
Figure 1. Oocyst excretion ($+$SEM; $n = 3$) per corticosterone group (no (———), from Day 16 (—— - -), from Day 25 (– – – –)) after repeated infection with 1a: 2.8×10^4 *Eimeria acervulina* starting on Day 11 or 1b: 2.8×10^3 *E. acervulina* starting on Day 11.

As shown in Figure 2, corticosterone administration had an immediate negative effect on body weight compared with untreated groups ($P < 0.0001$ from Day 18 onwards).
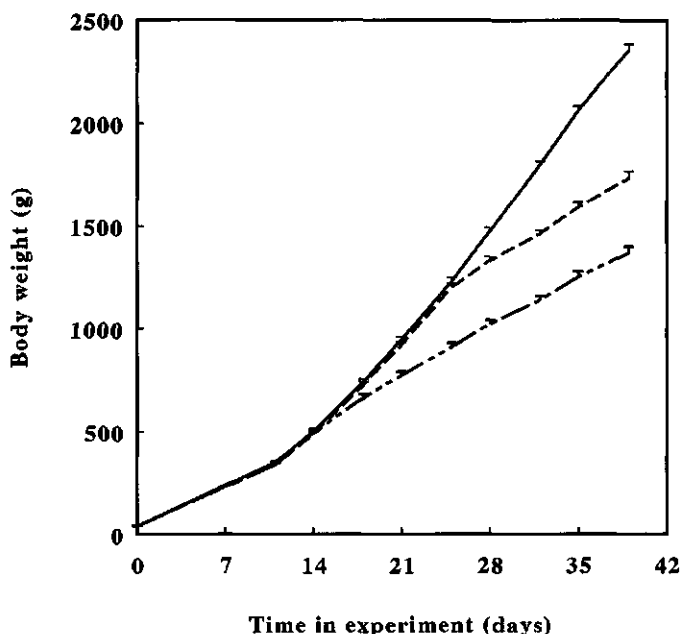
Figure 2. Body weight (+SEM; n=3) for the untreated (——) and corticosterone treated groups (from Day 16 (—— - -) or from Day 25 (— — — —)) averaged over infected and uninfected groups.

Figure 3 shows the effect of infection on body weight gain which is expressed as relative body weight gain. This was done since groups receiving infection had significantly higher body weights than groups receiving only placebo infection (tap water) before first infection had taken place ($P < 0.01$). Per animal the body weight gain in between measuring days, was calculated as percentage growth in a period relative to the body weight at the beginning of that period. Until Day 21, *i.e.* 10 days after initial infection, growth of both "low" and "high" infected animals was lower compared to uninfected animals (P varying from 0.0004 to 0.14). Between Days 28 and 33, body weight gain of the infected groups tended to be higher ($P=0.11$), indicating a phase of compensatory growth. There was no interaction effect of corticosterone and infection.

Effect of corticosterone on feed to gain ratio was significant ($P < 0.0001$ at Days 18, 21, 25, 28, 32, 35 and 39) and showed immediately after application (Figure 4). Effect of infection on feed to gain ratio was significant from Day 18 onwards (respectively $P < 0.10$ for Day 18 and $P < 0.005$ for following days), with higher feed to gain ratios in infected groups compared with uninfected groups (Figure 4). A significant interaction effect between

infection dose and corticosterone administration was present at Days 18, 21 and Day 25, *i.e.* 14 days after initial infection ($P <$ respectively 0.10, 0.001, 0.005) and at Day 32 ($P < 0.05$). This interaction effect was caused by a significant higher feed to gain ratio in "high" infected, corticosterone treated groups.
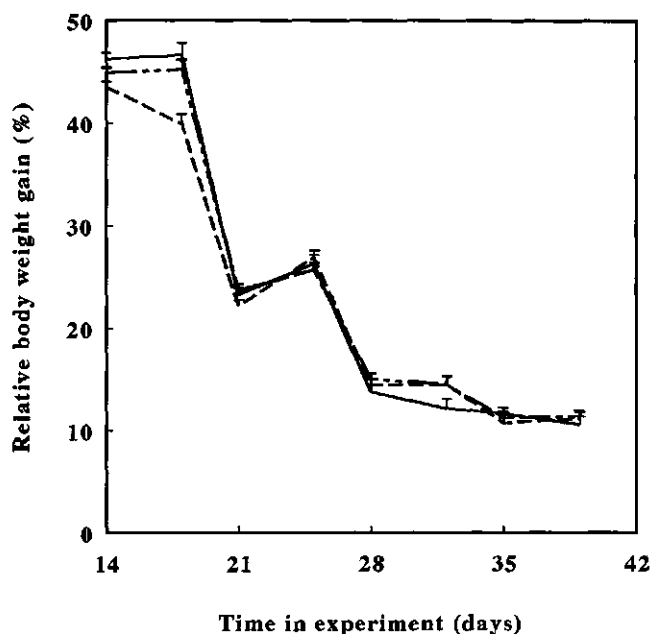
Figure 3. Relative body weight gain (+SEM; n=2 for uninfected and n=3 for infected groups) for each infection group (no infection (——); "low" (—— - -); "high" (– – – –)) as averaged over corticosterone treatments (Relative body weight gain in a period is calculated per animal as body weight gain in that period divided by the body weight at the start of that period multiplied by 100).

DISCUSSION

In the present experiment immune response was suppressed with corticosterone administration starting at 2 times during the period of oocyst excretion to check whether this immediately influences oocyst excretion. This was done to confirm the hypothesis that a time-lag may exist between first contact with the parasite and build up of protective immunity. This hypothesis resulted from a previous experiment (Graat *et al.*, submitted[b]), which was done for the evaluation of a computer simulation model (Henken *et al.*, 1994a,b).
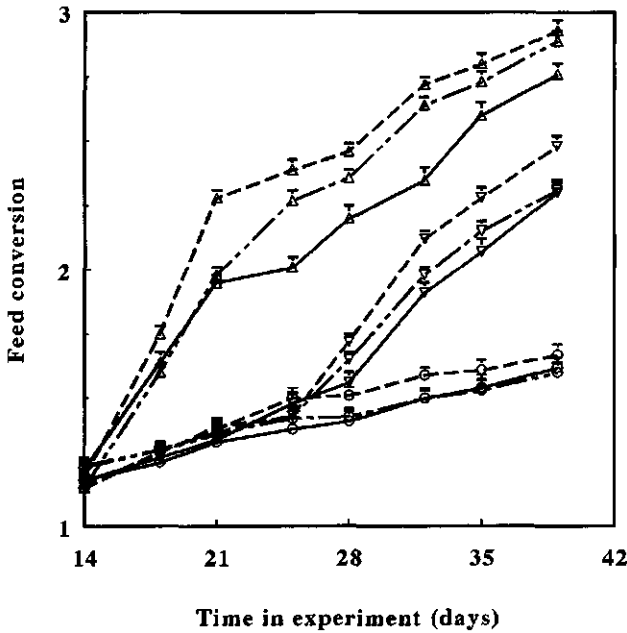
Figure 4. Feed conversion (+SEM; n = 2 if uninfected; n = 3 if infected) calculated from Day 11 (*i.e.* first infection day) for each treatment group (corticosterone: no (o), from Day 16 (▲), from Day 25 (▽); infection (no (——), "low" (—— - -), "high" (– – – –)).

If protective immunity already develops during the first days of infection, it might be expected that, because of immunosuppression, the peak of oocyst excretion would differ between treated and untreated groups. In the previous study corticosterone administration immediately affected lymphocyte responsiveness as tested with mitogen induced lymphocyte stimulation test (Graat *et al.*, submitted[b]). In the present experiment oocyst excretion was immediately affected by corticosterone, when administration started at Day 25, *i.e.* 14 days post initial infection (p.i.i.). When corticosterone treatment started at Day 16, *i.e.* 5 days p.i.i., oocyst excretion was immediately influenced in the groups infected with the "high" doses, but not in the groups infected with the "lower" doses. In the previous experiment (Graat *et al.*, submitted[b]) peak oocyst excretion was not affected in groups infected with the "low" doses, when corticosterone treatment started 4 days prior to infection. The combined results suggest that corticosterone treatment suppresses the effector function of protective immunity against *E. acervulina*, but does not prevent development of protective immunity itself. Results suggest that there is at least a time-lag between first infection and coming into effect of protective immunity.

Furthermore, in the computer simulation model it was supposed that velocity at which maximal acquired immunity is reached, depends on the amount of antigen

encountered. Therefore, in this study also the influence of the magnitude of infection dose on establishing protective immunity was studied.

Development of protective immunity depends on, amongst others, the parasite inoculation dose (Fernando, 1982; Rose, 1987), the developmental stage of the parasite and its mode of administration (Rose, 1987). Earlier results (Hein, 1968; Joyner & Norton, 1976; Long *et al.*, 1986; Stiff & Bafundo, 1993) showed that repeated immunisation with even very small numbers (5 to 20, 2×10^3) of *E. acervulina* is sufficient for (partial) protection against the negative effects on growth and feed to gain ratio independent of the age of the animals. In many of these experiments, ending of oocyst production was used as criterion of acquired immunity. Present experiment differed with these experiments in the application of corticosterone to induce immunosuppression.

Results show that the length of the time-lag depends to some extent on infection level as indicated by the differences between the "high" and "low" groups treated with corticosterone at Day 16 (5 days p.i.i). It seems that the rate at which immunity develops is slower in the "low" dose. The rate at which acquired immunity develops, at least in preventing intestinal parasite multiplication and thus in terms of oocyst production, might be very important with respect to damage and regeneration of intestinal cells. Results of present experiment imply that protective immunity developed faster in broilers repeatedly infected with 2.8×10^4 oocysts of *E. acervulina* compared with a ten times lower infection dose, at least when decline in oocyst excretion was regarded. In the higher dose a more pronounced peak was found followed by a sharp decline, in contrast with the lower dose where the peak spread over more days, followed by a more gradual decline. Therefore, it was suggested that at the lower infective dose, a longer time occurred for the rate at which immunity developed was maximal. Still, at both infection levels excretion stopped practically at the same days.

A possible explanation of a difference in time-lag might be a threshold in the number of antigen encountered (life-cycle stages: sporozoites, schizonts etc.) before the rate, at which immunity develops, becomes maximal. In the computer model it was suggested that a threshold exists. The threshold was set at 20,000, but this was based on sheer guessing since no information in literature could be found about existence of such a threshold. Therefore, the threshold assumption needs to be studied. Nevertheless, also with the lower dose immunity was acquired with a lower impact on production characteristics and agrees with other research (Hein, 1968; Long *et al.*, 1986; Conway *et al.*, 1993).

In conclusion, present and previous experiment (Graat *et al.*, submitted[b]) were done for the evaluation of a computer simulation model (Henken *et al.*, 1994a;b), with respect to aspects of build up protective immunity, namely time-lag and infection dose. Results suggest that there exists a time-lag between first infection and coming into effect of

protective immunity, and that infection level influences the rate at which protective immunity develops. Therefore, the results supported inclusion of these aspects in the computer simulation model.

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Effect of concurrent anticoccidial drug administration and corticosterone-induced immunosuppression on oocyst excretion, lesions and production of broilers infected with *Eimeria acervulina*

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Effect of concurrent anticoccidial drug administration and corticosterone-induced immunosuppression on oocyst excretion, lesions and production of broilers infected with *Eimeria acervulina*

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ABSTRACT

A study was made of the effect of concurrent monensin administration and cortico-sterone-induced immunosuppression on the development of immunity to E. acervulina in broilers. The hypothesis, derived from computer simulation, was that in E. acervulina infected birds with both a lowered immunoresponsiveness and lower anticoccidial drug efficacy, the negative effect on performance is more than additive compared to non immunocompromised animals treated with an effective anticoccidial drug. Immunisation in the prechallenge period was done with $\approx$ 30 oocysts (3 times weekly from Day 12 to 22). Oocyst output, lesions and production were measured during the prechallenge period until one week after challenge at Day 29 (1.6×10^5 oocysts).

Corticosterone resulted in a disproportional increased oocyst output with increasing drug levels. After challenge, previously corticosterone treated birds produced less oocysts and lesions. Before challenge, monensin showed a dose-response relationship resulting in lower excretion and lesions with higher drug dose. After challenge this was reversed. An interaction effect was found in lesion score after challenge with a smaller effect of corticosterone in 0 and 30 ppm monensin treated birds. These findings are in contrast with the hypothesis in which the largest corticosterone effect was expected with reduced drug efficacies, thus with lower drug dose.

Key words: coccidiosis, *Eimeria acervulina*, immunity development, anticoccidial drugs, broilers

INTRODUCTION

Infection with various *Eimeria* species remains a problem in intensive broiler meat production. Until now, the broiler producing industry relies on incorporation of

anticoccidial drugs in the feed. However, resistance to anticoccidial drugs has become a major problem (Chapman, 1993a). Resistance develops because of suboptimal levels of drugs together with the enormous reproductive capacity of the *Eimeria* species (Chapman, 1994). Sometimes, immunity development occurs in birds given anticoccidial drugs that do not fully suppress the parasitic life cycle (Chapman, 1994). This aspect is very important, since several days before slaughter a withdrawal period of the drug is obligatory in broilers. Numerous studies have been done testing development of immunity to coccidiosis with different anticoccidials. A varying effect of different classes of anticoccidials on immunity development has been found, varying from no effect to complete immunity development (Reid *et al.*, 1977; Chapman, 1978; Karlsson & Reid, 1978; Chapman & Hacker, 1993). However, all these experiments have been done in "normal" immunocompetent animals. Sensitivity analysis (Graat *et al.*, submitted[a]) of a simulation model describing *E. acervulina* infection in broilers (Henken *et al.*, 1994a,b) has led to the model hypothesis of an interaction between host immune status and anticoccidial drug efficacy. This interaction is reflected in a disproportional negative effect on growth and feed conversion by a slight decrease of immunity status in combination with a small decrease in anticoccidial drug efficacy. It is known that in poultry practice the presence of other diseases and stress might negatively influence the immune system (Siegel, 1995). Also, it is known that due to several factors, amongst which coccidiosis or other diseases, feed intake, and thus anticoccidial drug intake, might be affected. Also, the anticoccidial drug potency might vary due to the diet composition (Williams, 1992) and feeding regimen (Chapman, 1993b). Consequently, this could affect the efficacy of the used anticoccidial and the interference with the development of immunity against *Eimeria* spp. To test the model hypothesis of an interaction effect on production characteristics when both immune competence and anticoccidial efficacy are lowered, an experiment was conducted. Immunity development against *E. acervulina* was examined in chickens with a "normal" and by corticosterone "lowered" immuno-responsiveness together with varying doses of an anticoccidial drug.

MATERIAL AND METHODS

Chickens

One-day-old male chickens (n = 144; vaccinated for NCD) of a commercial strain (Hybro®) were used for the experiment. After arrival chicks were wingbanded and weighed.

Husbandry

Chickens were reared in wired floor cages (n = 24) until 36 days of age with 6 birds

per cage (0.6×0.75 m). At Day 10, chicks were divided in 6 weight categories (gram±SD: 235±9; 254±4; 267±3; 279±5; 294±4; 310±7). Each of these 6 groups consisted of 24 chicks. From each weight category one chick was randomly assigned to a cage, resulting in similar average weights per cage at the start of experimental treatments. Temperature at the start was set at 33°C and was declined to a minimum of 21°C at Day 26, which was continued till Day 36. Until Day 5 there was continuous light. From Day 5 onwards the lighting regime was a 23-h light and 1-h dark cycle. Standard commercial starter ration without anticoccidial drugs was given until Day 10 (ME=2973 kcal/kg). From Day 10 until Day 28 the chickens were fed a commercial grower ration with anticoccidial drugs and/or corticosterone according to the experimental design (ME=3024 kcal/kg). From Day 28 onwards anticoccidial drugs and corticosterone were withdrawn from the feed. Experimental diets and tap water were provided *ad libitum*.

Treatments

The study was conducted as a factorial arrangement of treatments with 2 levels of corticosterone (ICN Biomedical Inc.) in the feed (0 and 20 ppm), and 4 levels of anticoccidial drugs (0, 30, 60 and 100 ppm monensin) fed to chicks infected with *E. acervulina*, resulting in 8 treatment groups. The intended dose was 2.8×10^4 sporulated oocysts (drug sensitive strain from Houghton Poultry Institute, maintained and kindly provided by Dr. L. Maes of Janssen Pharmaceutica N.V.). Each chick received the infection dose contained in one ml tap water, from Day 12 onwards, every other day up to and including Day 22 directly in the crop with a 1-ml syringe. At Day 29 a challenge was given with 1.6×10^5 oocysts. The number of oocysts in the inoculum was determined with a haemocytometer (Fuchs-Rosenthal). Monensin was supplied as a premix (Elancoban) by courtesy of Mrs. Dr. J.H. van der Stroom-Kruyswijk of Elanco.

In the course of the experiment it became obvious something went wrong with the infection dose (based on results of control groups compared with those of control groups of previous experiments). Due to freezing in the refrigerator the actual dose was much lower as intended. Therefore, it was decided to give animals a challenge dose (1.6×10^5 newly obtained oocysts from the same strain) at Day 29. The dose that was applied until Day 22 of the experiment was tested afterwards in a small separate experiment. Six groups of four chickens were infected with (1) the "frozen" dose, (2) the challenge dose and dilution of it: (3) 1.6×10^4, (4) 1.6×10^3, (5) 1.6×10^2, (6) 1.6×10^1. Five days after infection oocyst output was determined and a line was fitted through the data ($R^2 = 98\%$) after which it could be deducted that the dose in the prechallenge period had been about 1000 times less (around 30 oocysts) than initially intended.

Corticosterone dose was based on an earlier experiment (Graat *et al.*, submitted[b]) and research of Gross *et al.* (1980). Monensin was chosen as anticoccidial drug since it allows some immunity development (Braunius, 1987; and personal communication). Effect on feed intake and weight gain at the recommended dose (100-120 ppm) can vary from no effect (Ward *et al.*, 1990; Damron, 1994) to a small effect (McDougald & McQuistion, 1980; Metzler *et al.*, 1987). With higher dosages (150 ppm) than recommended a significant decreased weight gain might be found, because of its depressive effect on food intake (Bartov, 1994). Considering above facts, monensin was used at 30, 60 and 100 ppm. Corticosterone and monensin were withdrawn one day before challenge of the animals.

Measurements

Birds were weighed individually at Day 0, and from Day 10 onwards every other day until Day 26, and on Days 29, 32, 34 and 36. Feed intake was measured per cage for the periods determined by the above mentioned weighing days. Feed intake was adjusted for mortality of chicks.

On Days 12, 16, 18, 20, 22, 24, 26, 29, 32, 34, 36 a mixed faeces sample of the preceding days was collected per cage and examined in duplo for oocysts using a McMaster counting chamber according to the method of Long & Rowell (1958). All faeces produced between measuring days was done by placing a weighed clean empty faeces reservoir beneath each cage and weigh it at the end of the period.

At Day 18 (*i.e.* 6 days after initial infection started), three chickens per cage (already randomly selected at Day 10) were killed by intravenous administration of T61 (Hoechst, München). Each chicken was checked on gender and abnormalities. Lesion scoring of the intestines was done according to Johnson & Reid (1970). At Day 36 all other chickens were killed and observed and examined as described for the chickens killed at Day 18.

Statistical analysis

Differences in treatments were determined by subjecting data (n = 24) to analysis of variance. The statistical model used (PROC GLM, SAS, 1989) for oocyst excretion, lesion scores, body weight, body weight gain, and feed to gain ratio, was:

$Y_{ijk} = \mu + A_i + C_j + (A \times C)_{ij} + e_{ijk}$,

where, μ = overall mean, A_i = effect of anticoccidial (i=0,1,2,3), C_j = effect of corticosterone (j=0,1), $(A \times C)_{ij}$ = interaction effect between anticoccidial and corticosterone, e_{ijk} = error term.

R E S U L T S

Oocyst excretion

Figure 1 shows the oocyst excretion as an average per animal during the experiment for each combination of anticoccidial drug dose and corticosterone. Broilers treated with corticosterone in the trickle period excreted higher numbers of oocysts at Day 18 ($P<0.10$), Day 20, 22, 24, and 26 ($P<0.05$). In the trickle infection period, excretion of oocysts showed a dose-response relation with monensin dose. With lower drug dosages a higher number of oocysts was excreted ($P<0.01$) at Days 18 up to Day 26. Application of corticosterone led to a disproportional increase in oocyst excretion with increasing drug dosages. This was reflected in a significant interaction effect ($P<0.01$) at Day 26. At other measuring days the interaction effect was not significant, but this is probably caused by the low number of observations (n=3 per group) and high variability in oocyst excretion.
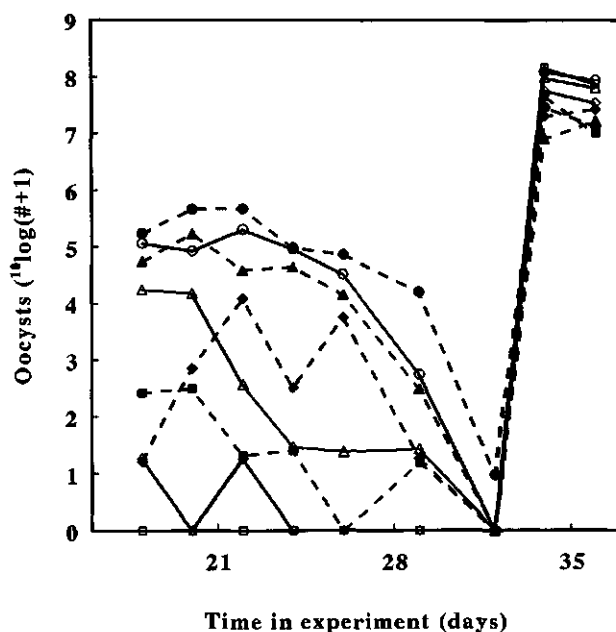
Figure 1. Oocyst excretion per chicken for each treatment group. (Corticosterone dose: none=open symbols and continuous lines; 20 ppm=closed symbols and dotted lines; Anticoccidial drug dose: none= ○,●; 30 ppm=△,▲, 60 ppm= ◇,♦ ; 100 ppm=□,■).

Challenge at Day 29 resulted in a high number of oocysts excreted in all treatment groups. Effect of previous anticoccidial drug application was not significant. However, a significant effect was present at Days 34 and 36 ($P<0.01$) with lower oocyst excretion for formerly corticosterone treated groups.

The total number of oocyst excretion in the post-challenge period was negatively correlated ($r = -0.47$; $P < 0.05$) with the number excreted in the pre-challenge period (trickle infection period).

Performance

Body weight gain (g/day) during trickle infection in corticosterone treated birds was significantly lower during the whole experiment ($P < 0.0001$) which resulted in significant lower body weights ($P < 0.0001$; Table 1). During the trickle infection period body weight gain was not significantly influenced by monensin application (Figure 2). However, after challenge growth was significantly decreased in previous monensin treated birds, especially for 60 and 100 ppm monensin ($P < 0.01$ and 0.05 for respectively growth from Day 29 to 34 and to 36). Body weight in the prechallenge period was affected by monensin application of 60 and 100 ppm as compared to 0 and 30 ppm from Day 22 onwards, although not statistically significant ($P \approx 0.15$).

Feed conversion was significantly influenced by corticosterone treatment ($P < 0.0001$) (Table 2). From Day 12 to 22 monensin at 100 ppm showed higher feed conversion than at other dosages ($P < 0.05$). At these days also the interaction between anticoccidial drug dose and corticosterone was present. This interaction was caused by disproportional higher feed conversion in the 100 ppm monensin and corticosterone treated groups. These latter groups also had the highest feed conversion after challenge, although not statistically significant.

Final body weight and feed conversion was best for groups treated with 30 ppm monensin, although not statistically significant.

Table 1. Least square means of body weight and SEM (g) at Day 10 (start experimental diets), Day 12 (first trickle infection), Day 18 (lesion scoring), Day 22 (last trickle infection), Day 29 (challenge), Day 34 and Day 36 (resp. 5 and 7 days after challenge).

Body weight (g) at Day		10	12	18	22	29	34	36
Corticosterone	0	273	343[a]	670[a]	922[a]	1419[a]	1735[a]	1872[a]
Dose	20	273	326[b]	514[b]	605[b]	738[b]	843[b]	931[b]
	SEM	1	2	6	8	21	22	25
Anticoccidial	0	273	333	586	770	1099	1324	1438
Dose	30	273	335	602	780	1096	1334	1462
	60	273	334	594	765	1067	1252	1350
	100	273	334	587	740	1051	1246	1357
	SEM	1	3	8	12	30	31	36

[a,b]Means within treatments with different superscripts differ significantly ($P < 0.0001$).
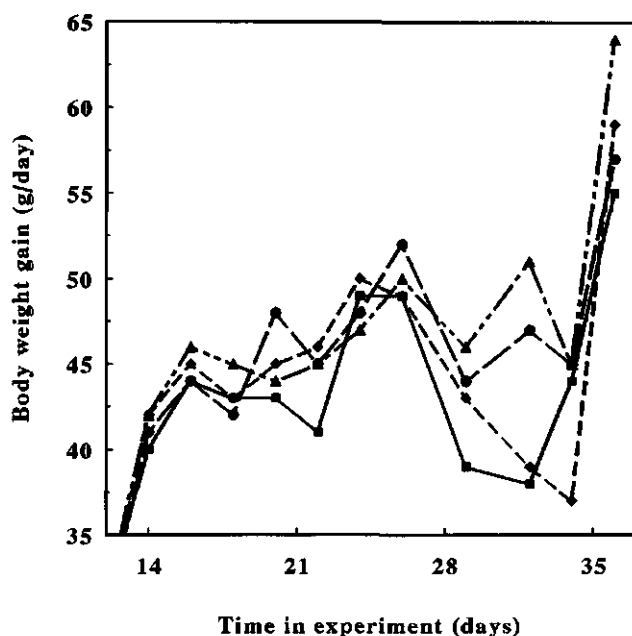
Figure 2. Least square means of body weight gain (g/day) in anticoccidial drug treatment groups. (Anticoccidial drug dose: ●–none; ▲=30 ppm, ◆ =60 ppm; ■–100 ppm) (SEM at the 12 measurement days is resp. 1, 1, 1, 1, 2, 3, 3, 4, 3, 3, 3, and 4 g).

Table 2. Least square means of feed conversion and SEM (g/g) from Day 10-12, Day 12-14, Day 12-18, Day 12-22, Day 12-29, Day 29-34 and Day 29-36 (Day 10=start experimental diets; Day 12=first trickle infection; Day 18=lesion scoring; Day 22=last trickle infection; d29–challenge); Days 34 and 36=resp. 5 and 7 days after challenge).

Feed Conversion (g/g) in period		10-12	12-14	12-18	12-22	12-29	29-34	29-36
Corticosterone	0	1.42^a	1.28^a	1.43^a	2.29^a	2.11^a	1.97	1.99
Dose	20	2.18^b	2.35^b	2.67^b	4.66^b	5.43^b	2.59	2.50
	SEM	0.04	0.01	0.04	0.04	0.26	0.26	0.26
Anticoccidial	0	1.85	1.80	2.10	3.41^A	3.54	2.14	2.22
Dose	30	1.79	1.82	2.00	3.40^A	3.70	1.83	1.85
	60	1.79	1.78	2.00	3.42^A	4.09	2.20	2.14
	100	1.78	1.87	2.10	3.65^B	3.76	2.93	2.75
	SEM	0.05	0.02	0.06	0.05	0.37	0.37	0.36

^{a,b}Means within treatments with different superscripts differ significantly ($P<0.0001$).
^{A,B}Means within treatments with different superscripts differ significantly ($P<0.05$).

Lesion scores

Figure 3 shows lesion scores at Day 18 and 36. Lesion scores at Day 18, *i.e.* 6 days after initial trickle infection were significantly ($P < 0.005$) higher in corticosterone treated groups. Effect of anticoccidial drug dose was also significant ($P < 0.005$) and resulted in higher scores at lower drug levels in a dose-dependent relation. At Day 36, however, the effect was reversed with significant lower lesion scores in previous corticosterone treated birds ($P < 0.0002$) and at lower drug levels ($P < 0.0001$). At Day 36, a larger effect of corticosterone was found in birds treated with 60 and 100 ppm than in birds treated with 0 and 30 ppm (Table 3). This interaction effect was significant ($P < 0.005$).
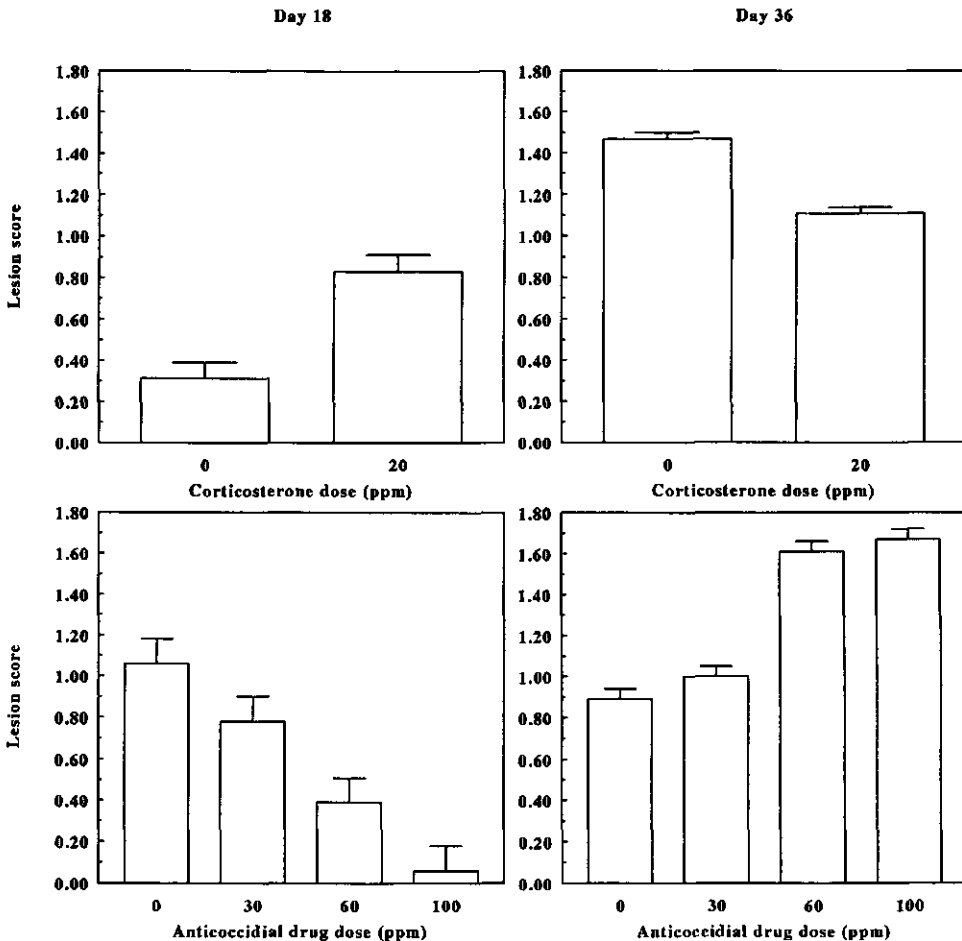
Figure 3. Least square means (+SEM) of lesion scores at Day 18 (6 days after initial trickle infection) and Day 36 (7 days after challenge infection) for corticosterone and anticoccidial drug dose.

Table 3. Least square means of lesion scores (SEM=0.1) at Day 36 for each treatment group.

Anticoccidial Dose (ppm)	Corticosterone Dose (ppm)	
	0	20
0	0.8	0.8
30	1.1	0.9
60	2.0	1.2
100	1.9	1.4

DISCUSSION

Several experiments have been carried out to study immunity development after infection with *Eimeria* spp., in combination with the effect of anticoccidials (Callender & Shumard, 1973; Reid *et al.*, 1977; Chapman, 1978; Karlsson & Reid, 1978; Long *et al.*, 1979; Chapman & Hacker, 1993). Protective immunity can be useful once medication is withdrawn from the feed. Immunity formation against *Eimeria* spp. measured in birds treated with different types of anticoccidials have been done in experiments with normal non immunocompromised animals (except for the coccidiosis infection) or in field studies. In a recently developed computer model simulating *E. acervulina* infection in broilers a high degree of interaction was found between level of immunocompetence of the host and level of anticoccidial drug efficacy (Graat *et al.*, submitted[a]). No experimental research has been done on this subject until now.

Experiments have been done with low immunising doses followed by challenge. Hein (1975) and Long *et al.* (1986) did experiments with immunising dosages ranging from respectively 2,000 (in combination with anticoccidial treatment) to 20,000 oocysts of *E. acervulina*. Two or more immunisations resulted in a more or less complete protection against the pathogenic effects of the challenge. Immunity development with these levels of infection is not age-dependent (Stiff & Bafundo, 1993). Joyner & Norton (1976) applied 5 or 20 oocysts for 25 days and found even with these low numbers of oocysts in trickle infection immunity formation. Chapman (1978) combined 13 inoculations with 10, 100 or 1000 oocysts of *E. maxima, E. brunetti,* or *E. tenella,* with monensin application (125 ppm). Immunity development in birds fed 125 ppm monensin was dependent upon the number of oocysts administered. All above mentioned experiments have been done in non immunosuppressed animals. Present experiment shows the concurrent effect of infection in immunocompromised birds treated with different anticoccidial drug levels.

Normal immunocompetent and with 100 or 60 ppm monensin treated birds showed a complete or almost complete absence of oocyst excretion during the prechallenge period.

In 0 and 30 ppm monensin treated groups a higher oocyst excretion was seen. This is as expected since with higher levels of drugs more sporozoites can take up the drug and therefore further development is inhibited, resulting in lower or no oocyst excretion (Long & Jeffers, 1982). Coccidial lesions in the prechallenge period were reduced with increasing monensin levels in a dose-dependent way as was found earlier (Reid *et al.*, 1972; Ruff *et al.*, 1976; McDougald *et al.*, 1996). After challenge however, more lesions were present in the previously 60 and 100 ppm monensin treated groups. This is also in agreement with earlier studies (Callender & Shumard, 1973; Reid *et al.*, 1977; Long *et al.*, 1979) where a dose-response relationship was found. The lower the dose, the better immunity can develop. The lower performance of 60 and 100 ppm birds in the prechallenge period, expressed in body weight (gain) and feed conversion compared to non-monensin and 30 ppm treated birds might be ascribed to the feed intake depression effect of monensin (Bartov, 1994). Considering body weight gain after challenge, it might be concluded that groups treated with 60 and 100 ppm monensin in the prechallenge period had developed lower protective immunity levels. This is especially obvious, since it is known that body weight gain increases significantly after withdrawal of monensin (McDougald & McQuistion, 1980; Metzler *et al.*, 1987). Thus, from combined results, it can be concluded that 60 and 100 ppm allowed less protective immunity to develop than the birds treated with 0 and 30 ppm and is in agreement with other studies.

In the prechallenge period, oocyst excretion was higher for corticosterone treated birds, in all drug treatment groups. This effect was also found in previous experiments (Graat *et al.*, submitted[b,c]). After corticosterone treatment stopped and a challenge infection was given, oocyst excretion was significantly lower in the previously corticosterone treated birds. This might be explained with a somewhat higher degree of protective immunity in those previously corticosterone treated birds. Corticosterone treated chickens probably encountered more stages of the life cycle of *E. acervulina*, including sporozoites, in the prechallenge period and with that more immunogenic stages resulting in a better triggering of the immune system. Jenkins *et al.* (1991a,b) found sporozoites to induce protective immunity. The results of present experiment appear to agree with the results of two earlier experiments (Graat *et al.*, submitted[b,c]), where it was concluded that corticosterone treatment particularly affects the effector function but not the development of protective immunity. This is supported by the lesion score results. Corticosterone treated birds in the prechallenge period had higher lesion scores. When lesion scores after challenge were considered, the results were completely reversed.

An interaction effect between anticoccidial drug dose and immunity status of the host was found in oocyst excretion. With corticosterone, a disproportional increase in oocysts output was found with increasing drug dosage. Lesion scores were very low in all

groups, both at a week after first infection in the prechallenge period, as after challenge. An interaction effect was found in lesion score after challenge, with a smaller effect of corticosterone in 0 and 30 ppm monensin birds. This might indicate that immunosuppression with corticosterone in birds which receive lower anticoccidial drug levels has less effect on immunity formation than with higher drug levels. These findings are in contrast with the hypothesis in which the largest corticosterone effect was expected with reduced drug efficacies, thus with lower drug dose.

The degree of acquired immunity due to the low trickle infection, as measured in performance, oocyst excretion and lesion score after challenge, was related to number of oocysts excreted in the trickle infection. Interpreting the total of results, it might be concluded that with a lower immunocompetence of the host, induced by corticosterone, in the initial trickle infections a higher degree of protective immunity is obtained finally when oocyst excretion and lesion scores are considered. Likewise, with a lower anticoccidial efficacy, a better immunity is obtained. An interaction effect was reflected in a lesser effect of immunosuppression with a lower anticoccidial efficacy.

The model hypothesis tested in this experiment was that in *E. acervulina* infected birds with both a lowered immunocompetence and with lower anticoccidial efficacy performance was disproportional worse than in normal non immunocompromised animals treated with an "effective" anticoccidial. From this experimental study, it could be concluded that the results were in contrast with this hypothesis. It would be interesting to repeat the experiment with higher trickle infection levels and make comparisons with naive chickens. Furthermore, immunosuppression was done by means of corticosterone. This might have been the wrong immunosuppressor to test the hypothesis, since it appears to affect the effector function but not development of immunity (Graat *et al.*, submitted[b,c]). Therefore, usage of immunosuppressors with a different mode of action than corticosterone might result in different outcomes.

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PART III

Risk of coccidiosis

Quantifying risk factors of coccidiosis in broilers using data of a veterinary poultry practice

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Quantifying risk factors of coccidiosis in broilers using data of a veterinary poultry practice

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ABSTRACT

A study was done to find and quantify risk factors for coccidiosis. The study population consisted of 4774 broiler flocks grown at 177 farms. Flocks were considered a case when at least one bird showed microscopical presence of oocysts in intestinal scrapings in a grow-out cycle. Other flocks were defined as controls. This was done for three types of *Eimeria*: *E. acervulina*, *E. tenella*, and *E. maxima*. Logistic regression was used to assess the relation between variables and the occurrence of *Eimeria* species. There were 49 variables, based on animal, flock or farm level. Univariate analysis was done for each variable. Variables with $P < 0.25$ were entered in a multivariate model. In the multivariate model only variables with $P < 0.10$ were kept in the model. In the final model farm-effect was taken into account. The results of the study showed an enhanced risk on coccidiosis due to environmental and management factors that increase the risk of introducing contamination or have a relation with hygienic measures (no use of overalls by visitors, type of farmyard which is difficult to clean, bad hygienical status, working personnel who might be working on other farms, presence of other animals on the farm, type of feeding and drinking system that is more difficult to clean). Also presence of diseases other than coccidiosis showed an increased risk. Most of these are already considered risk factors, however now they are quantified and could be used to put priorities for management advisory and intervention programmes to reduce losses due to the disease, or are helpful in developing risk management strategies.

Key words: coccidiosis, broilers, quantitative epidemiology, risk factor analysis

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INTRODUCTION

Coccidiosis in broilers is a world wide problem. Coccidiosis is caused by protozoa of the genus *Eimeria*. Effects of coccidiosis are a decreased body weight gain and a worsened feed to gain ratio, causing large economic losses (Braunius, 1987). In The Netherlands, these losses are estimated at 20 million Dutch Guilders (DFL) each year (1 DFL is approximately 0.58 US$). Costs associated with extra therapy and veterinary costs are not included (Voeten, 1987). It has been shown that eradication is not possible with an anticoccidial programme, due to increasing problems with drug resistance (Chapman, 1993). Use of vaccination programmes for commercial broiler meat production is not to be expected soon (Lillehoj & Trout, 1993). So, other ways of controlling the disease must be found. To develop an optimal prevention programme, knowledge about circumstances on farms is essential, enabling the identification of factors that significantly influence the probability of the disease occurrence. In the present study, factors associated with coccidiosis were quantified using data that covered a 3 year period and involved 177 farms, 11.4% of all broiler farms in The Netherlands. This study was done within the framework of building a simulation model for infection with *Eimeria* in broilers (Henken *et al.*, 1994a,b). The central point in this simulation model is the population dynamics of the parasite *E. acervulina* and effects of the parasite on broiler production. Effects of factors influencing both the parasite and host population and their interaction have to be implemented in the model. Analysis of data from broiler farms can provide knowledge on the relative impact of such factors facilitating future implementation into the model. Therefore, data from broiler farms were analysed to find and/or quantify these factors.

MATERIAL AND METHODS

General

In The Netherlands, broilers are raised from hatching (± 40 g) to slaughter (± 1850 g) in a flock cycle (grow-out) of about 6 weeks, with a feed to gain ratio of about 1.89. Broilers are raised at approximately 1550 farms with on average 30,000 broiler places. Each year about 7 grow-outs are realised (IKC, 1994).

Data

A database from a veterinary poultry practice was used. The database was intentionally designed to make use of up-to-date information to improve poultry flock health. The database contains, for each flock, information concerning anamnesis, clinical

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signs, therapy, vaccination, etc. that is collected by the visiting veterinarian (at flock level) and information collected in the laboratory (at animal level). The latter information regards results of dissections, bacteriology, drug sensitivity tests and serology. Data used in the present study were collected from January 1992 until January 1995. The primary data were derived from the database. Additional information was collected by a questionnaire, filled out by the veterinarians. The data were collected on 177 farms with 2.7 (SD = 1.8; range 1 to 10) houses (separate buildings) on average. Each farm had 11 grow-outs on average (SD = 6; range 1 to 21) in the database. In total, data from 4774 flocks are present in the database. This means that flocks are clustered within farms. In this study, a flock is defined as a growing period in one house. Between each grow-out, there is an empty period in which litter is removed from the houses and houses and equipment are cleaned. The farms in the study represent 11.4% of all Dutch broiler farms, but were mainly located in the southern part of The Netherlands, which reflects the major part of location of Dutch chicken industry. To see whether the farms in the database were representative for the Dutch broiler situation, farm sizes were compared. Figure 1 shows distribution of farm size in The Netherlands compared with the farm size in the database. From this figure, it can be seen that large farms (> 75,000 broiler places) are overrepresented. Very small farms (< 5,000) were underrepresented.

Definition of coccidiosis cases and variables

Ideally, a farmer sends once a week some chickens per flock for examination to the Poultry Practice. However, on average this was 3.4 times (range 1 to 15 times per flock). In the first 2 weeks of a flock cycle farmers sporadically send chickens. On average, 32 animals were send. This is an average of 5 chickens per time (range 3 to 21). These chickens were examined for the presence of oocysts in intestinal scrapings. Scrapings were examined microscopically (magnification 10 × 10). Ten fields of vision were counted, averaged, and given the label "control" if no oocysts were found. Since each *Eimeria* species is found at a specific site in the intestines, it is possible to distinguish between species. For each species *Eimeria* found, the label "minor", "moderate" or "serious", was given according to the classification given in Table 1. Four species of *Eimeria* were observed, *E. acervulina* type, which could include *E. praecox*, *E. mitis* and *E. mivati* (see Williams *et al.*, 1996), *E. tenella*, *E. maxima* and *E. brunetti*. However, the last species was only found in 0.1% (n = 3) of all flocks and was therefore omitted from the (statistical) analysis. For each species a flock was considered a coccidiosis case if at least one bird showed microscopical presence of oocysts during the flock cycle, irrespective of the degree of infection. Flocks in which no birds with microscopical presence of oocysts were found were regarded to be controls.
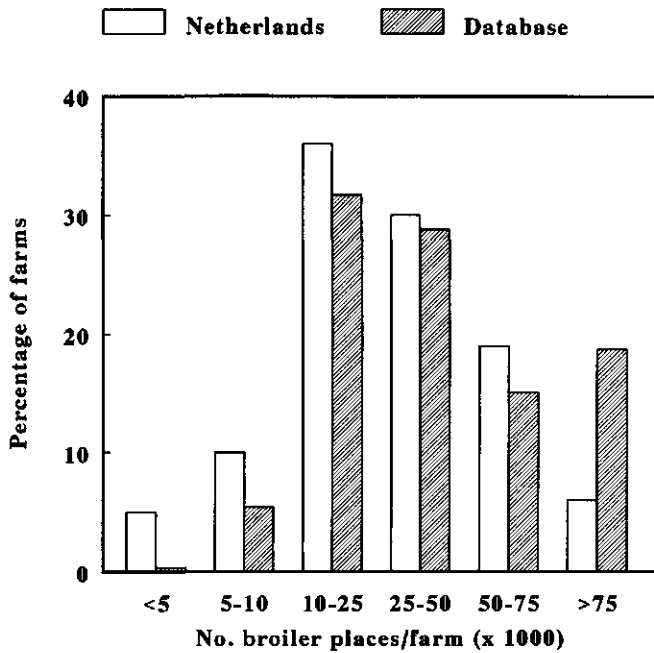
Figure 1. Distribution of farm size in The Netherlands in 1993 (IKC, 1994) compared with farm size in the database (mean = 46.6; SD = 41.2; range = 1.2 – 1,000).

Table 1. Classification of degree of infection with 3 *Eimeria* species.

E.acervulina	
Control:	: no oocysts
Case: minor	: = < 1 group of oocysts or = < 20 oocysts in case of singular oocysts
moderate	: 2-3 groups of oocysts or 21-50 oocysts in case of singular oocysts
serious	: > 3 groups of oocysts or > 50 oocysts in case of singular oocysts
E.maxima	
Control:	: no oocysts
Case: minor	: = < 10 oocysts
moderate	: 11-20 oocysts
serious	: > 20 oocysts
E.tenella	
Control:	: no oocysts
Case: minor	: = < 10 oocysts
moderate	: 11-30 oocysts
serious	: > 30 oocysts

Statistical modelling

Data analysis was done according to the case-control type of research. In a case-control study, a group of diseased units (cases; *i.e.* animals, flocks, farms) and a group of non-diseased units (controls) are selected and compared with respect to presence of the

hypothesized risk factor (Thrusfield, 1995). In this study, coccidiosis positive flocks were compared with coccidiosis negative flocks in relation to exposure to potential risk factors.

The strength of the association between the factor and occurrence of coccidiosis is calculated through an Odds Ratio (OR), an approximation of the Relative Risk. An OR greater than 1 indicates a positive statistical association between factor and disease, identifying the factor as a risk factor. An OR less than 1 indicates a negative statistical association: in that case the factor may be seen as having a preventive effect against the disease. An OR of 1 suggests that there is no association at all. The OR is significantly greater or less than 1 if the value 1 is not included in the confidence interval. The further the deviation from 1, the stronger the association between factor and disease is. Thus, the OR can be interpreted as the excess of risk of getting coccidiosis due to exposure to a certain factor.

Table 2. Variables taken into account in the statistical analysis

Variables[a] presented in categories

Coccidiosis control measures
In case of serious coccidiosis, in case of often occurrence of coccidiosis

Medication
Antibiotics, Anticoccidials, Vitamins, Others

Result of dissection other than coccidiosis
Locomotory disorders, Intestinal disorders, Pulmonary disorders, Other diseases

Chick house
Feeding system, Feeding scheme, Drinking water system, Control of drinking water with respect to contamination, Heating system, Ventilation system, Age of house, Floor condition, Climate regulation, Number of broilers per m^2, Measures to keep litter dry

Risk of introduction of diseases
Number of visits of veterinarian, Regular weighing of chicks during flock cycle, Working personnel, Admittance of visitors, Use of overalls by visitors, Hygienical status, ICC (Integrated Chain Control) commendable

Environment
Region, Other farm animals on the farm, Other broilers in neighbourhood, Presence of vermin, Destruction of vermin, Type of farmyard

Farmer characteristics
Age of farmer, Educational level of farmer

Others
Feed mill, Hatchery, Breed of chickens, Quality of faeces, Season, Coccidiosis status previous flock

[a]Most variables were categorical (see Tables 5-7).

First, univariate logistic regression was performed for each variable (Table 2) using presence/absence of each *Eimeria* species separately (PROC LOGISTIC: SAS, 1989). Variables associated with coccidiosis positive flocks at a 0.25 *P*-level, were used in multivariate logistic regression (for method see Hosmer & Lemeshow, 1989). In the multivariate model, variables were excluded from the model by a backward procedure. The least significant variable was deleted, the model refitted and results were compared with the previous run in order to check for confounding. With a change in parameter estimates of more than 30% change, the deleted variable was considered to be a confounder and included in the model again. This resulted in a model containing variables related to presence of coccidiosis (*P* < 0.10). For the multivariate logistic model the number of farms and flocks was decreased dramatically due to missing values (Table 3). In this multivariate model two-way interactions were tested for significance. Additionally, a NESTED procedure (Goelema *et al.*, 1991) was performed. In this procedure a random-effect term (farm as main plot) is included in the logistic regression model, as generally done in epidemiological studies in which units of analysis (flock) are clustered within for example a farm. In this study this was done because flocks on the same farm probably are not independent. NESTED will give an estimate of the variation between flocks that can be ascribed to variation between farms.

Differences in feed conversion and body weight gain between non-infected and infected flocks were calculated (PROC GLM, SAS, 1989). Feed conversion data were available from 1871 flocks only and body weight gain data from 645 flocks.

Table 3. Total number of farms and flocks (cases and controls) per *Eimeria* species as included in the multivariate logistic model.

Eimeria species	No. farms	No. flocks	No. cases	No. controls
E. acervulina	90	2178	1277	901
E. tenella	90	2177	676	1501
E. maxima	97	2499	211	2257

RESULTS

In 63% of all flocks one or more *Eimeria* species were found (Table 4). *E. acervulina* infection, alone or with other species occurred in 55% of all flocks. For *E. tenella* and *E. maxima* these percentages were respectively 31 and 10%. Fifty eight % of all positive flocks were contaminated with a single species. Feed conversion in positive flocks was 12% worse (*P* < 0.0001) than in control flocks. Daily body weight gain was 5% lower (*P* < 0.0001) in positive flocks.

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Of all farms, 92% was found positive for *E. acervulina*. This means that at least 1 grow-out had an infection with this species. For *E. tenella* and *E. maxima* there were respectively 84 and 47% positive farms.

Table 4. Number of positive flocks infected with *Eimeria* spp.

	No. of case-flocks	% of all case-flocks
E. acervulina	1407	46.5%
E. acervulina + *E. tenella*	836	27.6%
E. tenella	314	10.4%
E. acervulina + *E. tenella* + *E. maxima*	282	9.3%
E. acervulina + *E. maxima*	88	2.9%
E. tenella + *E. maxima*	56	1.8%
E. maxima	45	1.5%
Total positive flocks	3028 (63%)	100%
Total negative flocks	1746 (37%)	
Total flocks	4774	

Table 5. Explanatory variables with Frequency (%) and Prevalence (%) per Category and with Odds Ratios and 95% confidence interval of the NESTED[d] multivariate model for *Eimeria acervulina*.*

Variable related to:	Variable	Category	Freq	Prev	OR	95%.-CI
Medication:	Coccidiosis	No	94.8	55.7	Ref.	-
		Yes	5.2	86.4	2.9	1.5-5.6
	Others	No	47.7	53.0	Ref.	-
		Yes	53.3	61.1	1.4	1.3-1.7
	Vitamins	No	52.5	55.5	Ref.	-
		Yes	47.5	59.3	1.2	1.1-1.7[b]
Dissections disorders:	Locomotory	No	14.1	38.3	Ref.	-
		Yes	85.9	57.5	1.7	1.3-2.4
	Intestinal	No	4.1	27.2	Ref.	-
		Yes	95.9	56.0	1.9	1.0-3.7
	Pulmonary	No	16.5	39.0	Ref.	-
		Yes	83.5	57.9	1.6	1.2-2.1
Chick house:	Feeding system	Hanging tubes	62.0	50.6	Ref.	-
		Chain feeder	36.3	63.2	1.8	1.3-2.4
		Feeding pipeline	1.7	71.2	5.8	1.8-18.5
	Heating system	Tank-gas	60.7	56.1	Ref.	-
		Gas infra red heater	12.4	51.7	1.1	0.8-1.6[c]
		Central heating	6.3	57.0	1.8	1.0-3.1
		Radiant heater	5.5	54.5	665.6	35.6-12431
		Tank-oil	15.2	58.0	1.5	1.1-2.2

*Table 5 is continued on the next page.

Table 5. Continued

Variable related to:	Variable	Category	Freq	Prev	OR	95%.-CI
Risk of introduction:	No. of visits of vet.	Continuous parameter			1.1	1.0-1.3[b]
	Working personnel	No	83.6	53.5	Ref.	.
		Yes	16.4	66.8	1.4	0.9-2.1[b]
	Admittance of visitors	Just Veterinarian	45.1	53.8	Ref.	.
		+Advisor	52.0	57.6	1.4	1.0-1.8
		+Others	2.9	50.9	0.5	0.2-1.2[c]
	Use of overalls	Yes	96.7	56.6	Ref.	.
	by visitors	No	3.3	29.9	3.1	1.6-6.2
	Hygienical status	Good	94.3	55.1	Ref.	.
	(vet's opinion)	Not good	5.7	66.2	2.9	1.6-5.3
	ICC commendable	Yes	86.7	54.7	Ref.	.
		No	13.3	62.1	2.7	1.7-4.3
Environment:	Other farm business	No	57.7	52.5	Ref.	.
		Horticulture etc.	23.0	60.7	1.0	0.7-1.3[c]
		Other farm animals	19.4	59.2	1.6	1.2-2.2
	Presence of vermin	No	57.9	56.8	Ref.	.
		Yes	42.1	54.1	0.5	0.3-0.6
	Type of farmyard	Pavement	92.4	55.1	Ref.	.
		No paving	7.6	62.1	2.3	1.4-3.8
Farmer characteristics:	Age of farmer	20-30 years	10.2	56.3	0.5	0.3-0.7
		30-40 years	53.5	57.7	Ref.	.
		> 40 years	36.5	53.4	0.5	0.4-0.7
	Educational level	None	49.5	55.0	Ref.	.
		Yes, poultry related	22.1	55.0	2.0	1.4-3.0
		Yes, no poultry related	28.4	57.5	1.6	1.2-2.2
Others:	Feed mill	1[a]	16.0	56.9	2.3	1.5-3.5
		2	10.1	53.3	2.4	1.6-3.8
		3	26.3	49.8	Ref.	.
		4	24.0	57.7	1.9	1.3-2.8
		Others	23.6	56.6	1.7	1.2-2.5
	Hatchery	1[a]	30.8	51.7	Ref.	.
		2	28.2	57.4	1.5	1.0-2.2[b]
		Others	41.0	55.3	1.0	0.7-1.4[c]
	Quality of faeces	Normal	28.9	50.6	Ref.	.
		Changeably	20.9	53.3	1.2	0.9-1.6[c]
		Wet	2.2	83.9	7.8	2.9-20.9
	Season	Spring/Summer	52.0	56.7	Ref.	.
		Autumn/Winter	48.0	60.8	1.2	1.0-1.5
	E.ac in previous flock	No	68.7	20.8	Ref.	.
	in same house	Yes	31.3	53.5	1.6	1.3-1.9

[a]Since these data were confidential just numerical codes were used
[b]Only significant at P <0.10 level
[c]Non significant category of variable
[d]0.02% of non-explained variance is farm-effect (not significant)

In the multivariate logistic regression the following variables were significantly associated with *E. acervulina* positive flocks: variables related to feeding system, heating system, feed mill, hatchery, other diseases (locomotion, intestines, pulmonary system), curative medicine application against coccidiosis or other diseases, presence of other farm

animals, age and education of farmer, quality of faeces (wet), bad hygienical status, not ICC commendable, admittance of visitors, no use of overalls by visitors, working personnel besides family, number of visits by veterinarian, non paved farmyard, season (autumn & winter), and *E. acervulina* in the previous flock (Table 5).

Table 6. Explanatory variables with Frequency (%) and Prevalence (%) per Category and with Odds Ratios and 95% confidence interval of the NESTED[d] multivariate model for *Eimeria tenella*.

Variable related to:	Variable	Category	Freq	Prev	OR	95%.-CI
Medication:	Coccidiosis	No	94.8	30.6	Ref.	-
		Yes	5.2	89.7	8.3	4.1-16.7
	Others	No	47.7	28.2	Ref.	-
		Yes	53.3	38.5	1.6	1.3-2.1
Dissections disorders:	Locomotory	No	14.1	12.8	Ref.	-
		Yes	85.9	34.2	3.4	2.1-5.5
	Intestinal	No	4.1	7.7	Ref.	-
		Yes	95.9	32.2	4.4	1.0-20.5[b]
	Pulmonary	No	16.5	16.9	Ref.	-
		Yes	83.5	34.0	2.4	1.6-3.5
Chick house:	Heating system	Tank-gas	60.7	33.1	Ref.	-
		Gas infra red heater	12.4	23.4	0.9	0.5-1.4[c]
		Central heating	6.3	37.5	2.6	1.4-5.1
		Radiant heater	5.5	25.5	1.6	0.7-3.6[c]
		Tank-oil	15.2	31.2	0.8	0.5-1.3[c]
Risk of introduction:	Admittance of visitors	Just Veterinarian	45.1	25.8	Ref.	-
		+Advisor	52.0	33.4	2.9	1.7-4.6
		+Others	2.9	31.0	1.6	0.5-4.7[c]
	Use of overalls	Yes	96.7	30.5	Ref.	-
	by visitors	No	3.3	13.4	2.9	1.0-8.7[b]
	ICC commendable	Yes	86.7	28.6	Ref.	-
		No	13.3	38.5	1.9	1.2-3.0
Farmer characteristics:	Educational level	None	49.5	28.7	Ref.	-
		Yes, poultry related	22.1	28.0	1.3	0.8-2.1[c]
		Yes, no poultry related	28.4	33.5	4.1	1.6-10.7
Others:	Hatchery	1[a]	30.8	22.7	Ref.	-
		2	28.2	36.1	3.2	1.9-5.3
		Others	41.0	34.1	2.3	1.4-3.7
	Season	Spring/Summer	52.0	29.8	Ref.	-
		Autumn/Winter	48.0	32.4	1.2	1.0-1.5[b]
	E.te in previous flock	No	41.1	44.2	Ref.	-
	in same house	Yes	58.9	68.7	2.4	1.9-3.1

[a]Since these data were confidential just numerical codes were used
[b]Only significant at P<0.10 level
[c]Non significant category of variable
[d]1.6% of non-explained variance is farm-effect (not significant)

For *E. tenella* the following variables were significantly associated with positive flocks: variables related to heating system, other diseases (locomotion, intestines, pulmonary

system), curative medicine application against coccidiosis or other diseases, education of farmer, bad hygienical status, not ICC commendable, admittance of visitors, use of overalls by the visitors, hatchery, season (autumn/winter), and *E. tenella* in the previous flock (Table 6).

Following variables were significantly associated with *E. maxima* flocks: variables related to coccidiosis control (extra measurements if coccidiosis occurs too often), other diseases (pulmonary system), curative medicine application against coccidiosis, bad hygienical status, not ICC commendable, number of visits by veterinarian, feed mill, season (autumn/winter), and *E. maxima* in the previous flock (Table 7).

Table 7. Explanatory variables with Frequency (%) and Prevalence (%) per Category and with Odds Ratios and 95% confidence interval of the NESTED[d] multivariate model for *Eimeria maxima*.

Variable related to:	Variable	Category	Freq	Prev	OR	95%.-CI
Coccidiosis control	Extra measures if cox occurs too often	Does not occur	30.3	7.4	Ref.	-
		Close monitoring/hyg.	61.0	8.2	1.3	0.6-2.8[c]
		Treatment	8.6	35.9	4.2	1.4-12.4
Medication:	Coccidiosis	No	94.8	9.5	Ref.	-
		Yes	5.2	36.4	2.8	1.3-5.8
Dissections disorders:	Pulmonary	No	16.5	5.5	Ref.	-
		Yes	83.5	10.8	2.3	1.2-4.6
Chick house:	Measures to keep litter dry	None	9.7	3.4	Ref.	-
		Water restriction	27.3	5.1	4.0	0.9-17.8[b]
		Climate	8.2	10.3	11.5	2.4-53.3
		Combination	54.8	12.7	5.2	1.2-23.4[c]
Risk of introduction:	No. of visits of vet.	Continuous parameter			1.2	1.0-1.5
	Hygienical status (vet's opinion)	Good	94.3	7.6	Ref.	-
		Not good	5.7	41.7	38.2	2.1-694.6
	ICC commendable	Yes	86.7	8.0	Ref.	-
		No	13.3	19.8	135.3	7.4-2478.8
Others:	Feed mill	1[a]	16.0	10.2	0.7	0.3-1.6[c]
		2	10.1	8.9	0.4	0.2-1.2[c]
		3	26.3	14.6	Ref.	-
		4	24.0	5.8	0.3	0.1-0.6
		Others	23.6	9.0	0.2	0.1-0.4
	Season	Spring/Summer	52.0	8.5	Ref.	-
		Autumn/Winter	48.0	8.8	1.5	1.0-2.2
	E.ma in previous flock in same house	No	90.7	3.5	Ref.	-
		Yes	9.3	58.4	11.4	6.9-18.8

[a]Since these data were confidential just numerical codes were used
[b]Only significant at *P*<0.10 level
[c]Non significant category of variable
[d]7.0% of non-explained variance is farm-effect (*P*<0.05)

Flocks on rough floors (*vs.* smooth) had a slightly increased risk on all types of *Eimeria*, although not significantly. This factor was kept in the model since deletion led

to more than 30% change in parameter estimation and therefore considered a confounder.

A significant farm-effect due to clustering of flocks within a farm, was absent for *E. acervulina*. When the variable "coccidiosis in previous flock" was not included in the model the percentages of non-explained variance in the models for *E. tenella*, and *E. maxima*, were respectively 33% and 40% (both $P<0.0001$). However, after including this variable, these figures decreased to respectively 1.6% ($P>0.10$) and 7.0% ($P<0.05$), indicating the importance of this variable. For *E. acervulina* this percentage declined from 1 to 0.02%.

DISCUSSION

This study was done to find and quantify potential risk factors of coccidiosis in broilers. Representativeness of outcomes for total Dutch broiler farms may be decreased, since farms were concentrated in the southern part of The Netherlands. It should be stressed, however, that the major part of broiler farms is located there. Further, it should be emphasised that a flock was considered positive if one animal per grow-out showed oocysts, irrespective of the degree of infection. Due to large variation in times and number of chickens per flock that were send to the poultry practice, the degree and course of an infection were not included in the analysis. Therefore, the definition of case flocks could be criticized. However, with this definition, *Eimeria* positive flocks showed worse feed conversion and body weight gain than negative flocks, justifying the applied definition.

Flocks grown at one farm are assumed more alike than those grown at another farm, since management and environment of flocks is practically identical. Likewise, flocks in the same house are not independent, reflected in an increased risk if the previous flock was positive. To conclude flocks were clustered within houses, and houses within farms. Clustering within farms was accounted for in the statistical analysis. Correction for within house clustering was not possible, due to the large variation in flocks per house.

This study agrees with others that *E. acervulina* type is the most prevalent *Eimeria* species in broilers (Braunius, 1987; Voeten, 1987; Williams *et al.*, 1996). Infection occurs more often in autumn/winter as was found by Braunius (1985). Extra medication, additional to routine incorporation in the feed, was associated with presence of coccidiosis. In contrast to findings of Henken *et al.* (1992b), presence of other disorders were associated with occurrence of coccidiosis. In a study of Giambrone *et al.* (1977) broilers became more susceptible to *E. tenella* after infection with infectious bursal disease (IBDV). Many experiments have been done to show interaction between *Eimeria* and *Salmonella* (Takimoto *et al.*, 1984; Tellez *et al.*, 1994; Qin *et al.*, 1995). Ruff (1989) reviewed the interaction with bacteria (*E. coli*, *Salmonella*), viruses (Marek's disease, IBDV, reticuloendothelial

virus) and mycotoxins. Since coccidial species are present ubiquitous, it is important to know about their relations with other diseases, especially if coccidial infections are predisposing for zoonoses (*e.g. Salmonella*).

The results of present study showed an enhanced risk on coccidiosis due to environmental and management factors which might facilitate introduction of the parasite or have a relation with hygienic measures (no use of overalls by visitors, type of farmyard which is difficult to clean, bad hygienical status, not ICC commendable, working personnel who might be working on other farms, presence of other animals on the farm, type of feeding and drinking system which is more difficult to clean). Most of these are already considered risk factors, however now they are quantified and could be used to put priorities for management advisory and intervention programmes to reduce losses due to the disease.

Currently, emphasis is placed on developing new animal health/risk management strategies. In these strategies risks of introduction, transmission and emission of pathogens within and between farms have to be identified and quantified (Noordhuizen & Welpelo, 1996). Although the motive for need and development for such programmes has its origin in preventing zoonoses, they are useful for reducing occurrence of all diseases and improving animal health in general. The state of herd health is not only affected by the farmer's management. Also all goods (*e.g.* feedstuffs) and services (*e.g.* veterinarian) are potential risks for the introduction of disease agents (Noordhuizen & Welpelo, 1996). *Salmonella* in broiler breeders was associated with some feed suppliers (Henken *et al.*, 1992a). In present study number of visits of a veterinarian and purchase of food from some suppliers were related to coccidiosis and probably to other diseases. Differences between feed suppliers in this study might be related to anticoccidials incorporated in feed and timing of rotation to other anticoccidials. Since these data were confidential, this information was missing. In health control of farms, cooperation of suppliers should thus be mandatory as stated by Noordhuizen & Welpelo (1996). These authors also mentioned problems in implementing such programmes in practice due to farm-specific risk factors. With *E. maxima* infections a farm-effect was present. So, other factors than investigated contribute to the presence of coccidiosis and therefore more research is needed.

In this study, data of almost 50% of flocks could not be used in the statistical analysis. This is one of the problems with routinely collected computerized data (Thrusfield, 1995). Careful data collection in the assessment of risk factors is very important, especially in multivariate logistic regression where missing information on only one variable in the model will lead to loss of the total record.

In a case-control study it is difficult to demonstrate causality (Rothman, 1986). This was obvious in this study with *e.g.* following variables: association of coccidiosis with the presence of other diseases, wet litter, and number of visits of veterinarian. Unexplainable

or 'strange' associations (*e.g.* radiant heaters) might be due to random error (especially if a large number of factors is screened), confounding or imperfect biological knowledge. Therefore, the subsequent step should be to reveal the causal mechanisms by experimental work.

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General Discussion

INTRODUCTION

Considering the problems in the control of coccidiosis and limitations of alternative strategies (see General Introduction), a research project was started to model *Eimeria acervulina* infections in broilers. The objective of the project was to increase understanding of factors which influence the dynamics and mechanisms of an *Eimeria* infection in broilers and its effect on production. Theoretical, experimental, and field work were integrated. First, a model was developed. Experimentation with the model led to determination of experiments with the system (*i.e. Eimeria* infection in broilers) to validate principles and phenomena found in simulation results. The main objective of the research described in this thesis was focused on (qualitative) validation of the simulation model. Ideally, the project should contribute to a better decision basis for improving control strategies.

In Part I, a simulation model of *E. acervulina* infections was formulated based on knowledge from literature, or, if unknown, based on best estimates. The model served to detect lacking knowledge relevant to understand infection dynamics. It helped to formulate experimental and observational research. Experiments (Part II) were aimed at estimation of missing or, less known, important quantitative or qualitative aspects of the life-cycle of the parasite (Chapter 2.1) and their effects on production. Chapter 1.1 gives a description of the population dynamics of the parasite. It was assumed that the parasite is always present in broiler houses. To what extent this assumption is true and which factors affect the risk of infection of broiler flocks is described in Part III. Chapter 1.2 depicts the relation between the population dynamics of the parasite and production characteristics of broilers. The most important aspect of this part of the model was the derived hypothesis of an optimal initial contamination level. This hypothesis was explored experimentally (Chapter 2.2). Sensitivity of the model's behaviour to changes in parameter values was described in Chapter 1.3. Results of this sensitivity analysis led to some more hypotheses concerning immune status of the host and anticoccidial drug efficacy which both were tested experimentally (Chapters 2.3 to 2.5).

In this chapter, first the choice of simulation model is dealt with. Since this thesis was focused on validation the importance of validation as a process in modelling is discussed too. Subsequently, results of experiments are considered with respect to what extent the validation was successful. Feasible improvements of the current model are outlined, leading to directions for further research. Finally, some practical implications and concluding remarks are given.

CHOICE OF MODEL

Modelling coccidiosis in broilers is not a simple task because of the complex nature of this infection. First, infection can occur with 9 different *Eimeria* species, being tissue specific and having different immunogenic and pathogenic properties (Fernando, 1990). The immunogenic property is reflected in a varying degree of immunity after exposure to primary infections with different *Eimeria* species. *E. maxima* and *E. brunetti* are the most immunogenic species. *E. acervulina*, *E. mitis*, and *E. praecox* are moderately immunogenic, and *E. tenella* and *E. necatrix* are the least immunogenic (Ovington *et al.*, 1995). Pathogenic differences among *Eimeria* species result in a spectrum of negative effects on production characteristics after infection (Hein, 1976). Second, besides immunogenic and pathogenic differences between species, each field strain of an *Eimeria* species might have different immunogenic and pathogenic features (Wallach *et al.*, 1990; Shirley, 1992). Because of this antigenic diversity, immunity induced to one strain of a species does not necessarily protect (fully) against challenge with another strain of the same species (Shirley, 1992). Even if it is not possible to formulate a model comprising all these aspects simultaneously, modelling in itself is instructive for understanding the complex nature of the problem. Because of the complex nature of coccidiosis it was decided to start with a model simulating only infections with *E. acervulina*, since this is the most prevalent species in the world (Williams *et al.*, 1996).

It was decided to start with a dynamic deterministic model, since these are more useful in enriching general theories. The potential for applications to real data, however, is less than for stochastic models. Deterministic models have more attractive model formulations than the model equations in stochastic models, which often are difficult to solve in terms of explicit and manageable expressions (Becker, 1989). Deterministic models can reveal more clearly what the important characteristics are, because the important characteristics are often mixed with less important ones (Becker, 1989). In stochastic modelling demonstration of important characteristics is also possible, but then a careful design of experiments is essential for reliable results (Kettenis, 1994) and it is much more laborious.

Anderson & May (1991) made a distinction between microparasites and macro-parasites. Microparasites are those parasites that reproduce within the host. Most viral and bacterial parasites and many protozoan and fungal parasites belong to this category. The general assumption in modelling this type is that the host acquires life-long immunity that does not depend on the severity of infection, and that infection is of short duration compared with the life-span of the host. The <u>host</u> is central unit of modelling and is only modelled qualitatively (*e.g.* Susceptible, Infected, Recovered/immune: SIR-models). Macro-

parasites are those having no direct reproduction within the host, with generation intervals often being a considerable fraction of the hosts' life span (*e.g.* most parasitic helminths). When an immune response is elicited, it usually depends on the past and present number of parasites harboured by the host, and it tends to be of a relatively short duration.

In coccidiosis in broilers, the elicited immune response and pathogenic effects depend on the encountered number of parasites, and duration of infection is relatively long (approximately 25% of the hosts' life-span), as in macroparasites. In the short life of broilers, acquired immunity can be life-long, as in microparasites. Changeover from Susceptible to Immune is a more continuous process and quantitatively dependent on the number of ingested oocysts during infection (Braunius, 1987). So, although coccidiosis might be considered as an intermediate between the two types, it was chosen to model it as a macroparasite. Here, the parasite is unit of modelling rather than the host. Results described in Chapter 2.5 confirm the quantitative nature of *E. acervulina* infection and hence support the choice of a macroparasite type of model. In our simulation model population dynamics of *E. acervulina* was described quantitatively and negative effects on the host depended on the number of parasite stages encountered. Model description and behaviour were described in Part I and led to further hypotheses to be tested in experiments.

QUALITATIVE VALIDATION

Validation of a model is crucial in simulation studies (Kettenis, 1994). Validation is the process of bringing the user's/maker's confidence, that any inference about a system derived from simulation is rather correct, to an acceptable level (Shannon, 1975). Testing validity of the model has to be done by comparing model behaviour with (experimental) results (Kettenis, 1990). When these agree over a wide range of different circumstances, some confidence in the understanding of the system is obtained. If not, needs for additional or more reliable experimental data to estimate parameters more precisely are highlighted (Black *et al.*, 1993). Figure 1 in the General Introduction showed a schematic concept of a modelling cycle (Kettenis, 1990). With evaluation of a model, that is comparison of simulation results with "real world" results, it can be shown that, given the objectives, the model might be a good acting representation of the system under study. This is the only way to escape from the model cycle, because a model will never behave the same as the real system. It is good to emphasize that models, however complex they may be, always are simplified representations of the "real world" systems.

Part II of this thesis dealt with experimental validation of parameter estimates (Chapter 2.1) or hypotheses derived from model simulation (Chapter 2.2 to 2.5). An

experiment was done to estimate parameters with regard to the sporulation process. Sensitivity analysis showed that changing sporulation rate did not lead to substantial changes of simulation results. However, since experts in coccidiosis presumed climatic and litter conditions very important in facilitating infection with *Eimeria* spp., it was decided to study sporulation under circumstances encountered in practice. The results of the experiment showed that sporulation percentage is not the most important factor, since results were almost equal for all tested environmental conditions. So, both in the model and in simulated practical conditions sporulation rate proved to be of minor importance.

Qualitative validation was done for the model as a whole (Chapter 2.2) or for parts of the model (Chapter 2.3 to 2.5). This approach suits well in demonstrating principles or phenomena, however not for accurate prediction of outcomes. For educational aims quantitative agreement is less important. So, since the simulation model was mainly developed to improve understanding of development and effects of coccidiosis, this method of demonstrating principles is adequate. Table 1 summarises the qualitative and quantitative agreement between simulation and experimental results for those aspects that were tested.

Table 1. Qualitative and quantitative validity of a model simulating *E. acervulina*.

| | Agreement | | Chapter |
Item	Qualitative	Quantitative	
Model as a whole:			
Optimum infection level	Yes	No	2.2
Parts of model:			
Total immunity	Yes	No	2.3
Aspects of immunity:			
TIMELAG[1]	Yes	Yes	2.3 & 2.4
MAXIMM	---[2]	---	
MAXGRO	---	---	
INNATE	---	---	
CUMIMM	Yes	No	2.4
Interaction anticoccidial efficacy and total immunity	No	No	2.5

[1] TIMELAG = time-lag between parasite contact and effect on immunity; MAXIMM = maximum level of immunity attainable after exposure; MAXGRO = maximum development rate from innate to maximum immunity; INNATE = natural level of immunity; CUMIMM = cumulative number of damaged mucosa cells as a representation of the amount of antigen encountered and thus involved in immunity development.
[2] --- = validation has not been done.

In the experiment described in Chapter 2.2, validation was carried out for the model as a whole to obtain evidence supporting the hypothesis of an optimal initial contamination

level with respect to broiler productivity. The results showed that from a qualitative perspective the simulation model behaves realistic for those initial contamination levels tested. It was, thus, confirmed that there is an optimum infection level at which economic loss is lowest as compared to non-infected flocks. Quantitative agreement between simulation and experimental results was less satisfactory. The degree and rate at which oocysts are ingested from the litter could not be determined in this study, but might be an important aspect with respect to quantitative comparison. Rate of intake of oocysts fell in the range used by Parry *et al.* (1992), the only authors who made an estimation of the uptake of oocysts from the litter.

Two experiments were conducted to validate the model with respect to parameters of the broilers' immunity status. In the first experiment (Chapter 2.3) it was validated that with a lowered immune responsiveness the effects of an *E. acervulina* infection on broiler production were much larger than in non-immunocompromised birds. Overall, the results of this experiment showed that the simulation model qualitatively agreed with experimental results. However, in the simulation model, effects of differences in general immune responsiveness appear to overestimate the effects as were observed in the experiment. A time-lag of 120 hours between establishment of infection and immunity development was assumed in the simulation model; experiments (Chapter 2.3 & 2.4) supported inclusion of this aspect in the simulation model.

Sensitivity analysis of the simulation model showed that with both a lowered immunocompetence of the host and a lowered anticoccidial efficacy, performance of broilers is disproportionally worse than in normal non-immunocompromised animals treated with an effective anticoccidial drug dose. A study was made to test this hypothesis (Chapter 2.5). The largest effect of immunosuppression was found with higher drug levels. These findings were in contrast with the hypothesis in which the largest immunosuppressive effect was expected with reduced drug efficacies. Immunocompromised birds developed a better immunity to *E. acervulina* than non-immunocompromised birds. Immunosuppression was done by means of corticosterone. This might have been the wrong immunosuppressor to test the hypothesis, since it appears to affect the effector function, but not immunity development. Therefore, immunosuppressors with a different point of action than corticosterone might result in different outcomes. Further, the fact that the applied infection dose was very low, also may have contributed to the surprising results mentioned above. Clearly, more attention needs to be focused towards this area of research.

Model validation often is difficult to accomplish (Black *et al.*, 1993; Kettenis, 1994), especially when highly contagious or diseases with international trade bans (such as Newcastle Disease) are simulated and therefore experimenting is less possible. Even when it is possible to experiment with the system, as it is in coccidiosis in broilers, the fact that

a model behaves accurately under one set of circumstances does not mean that it is valid. However, the wider the circumstances under which the model behaviour is accurate, the more confidence is developed in the appropriateness of the concepts and parameters upon which it is based and the more useful will be its predictions. Model evaluation depends upon the existence of accurate results from experiments and complete description of experimental conditions to which the model is sensitive. Besides the fact that with doing many experiments the researcher obtains a better understanding of the system, he/she will make much progress. In fact, the purpose of simulation is experimenting with effects of varying conditions in the simulation model without the need repeating them in the "real system". Martin *et al.* (1987) stated that subsections of a model should be validated as separate exercises, prior to validation of the model as a whole. However, Black *et al.* (1993) reported that many factors might interact and that the consequences of manipulation of individual components of the system can be resolved only qualitatively. The experiment described in Chapter 2.2 was done for the model as a whole, without manipulating chickens. The experiments described in Chapter 2.3 to 2.5 were also done for the model as a whole, although now the chickens' immune system was manipulated. So, both approaches of Martin *et al.* (1987) and Black *et al.* (1993) were done and found useful.

As stated by Van Dam (1995) the traditional approach in studying production problems is empirical. Experiments are done, while varying the factor of interest and keeping all other conditions equal. Models derived from this type of study are data-driven and only descriptive. Explanatory models are theory-driven. In these models available knowledge is integrated leading to a better understanding of the systems' behaviour. A comparison of predicted system behaviour (simulation results) with observations (experimental results) helps to identify gaps, resulting in research geared specifically at filling these gaps (theory-driven). When validating the model as a whole, it is possible to find realistic outcomes with a certain input. However, underlying mechanisms might be totally erroneous. Manipulation of the chickens' immune states showed qualitative agreement and supported inclusion of some aspects in the model. However, underlying mechanisms remain unclear.

Since it is not possible to validate a model for all conditions, qualitative validation can increase confidence in the model (Kettenis, 1990). Considering Figure 1 in the General Introduction, it can be seen that the research done until now was concentrated on the upper part of the Figure. Only qualitative agreement between model outcomes and experimental results was shown for some elements (Table 1). Results of the experiments provided some confidence in the model as a whole. Nevertheless, still a lot of work has to be done to adjust and improve the current model.

IMPROVING CURRENT MODEL

Improvement of the current model is feasible for several aspects. Some of them will be discussed briefly in the following paragraphs. In this thesis, aspects of immunity, anticoccidial drugs, and external factors were studied more intensively and are therefore discussed in separate sections.

Spread of infection through a broiler house is not incorporated. It was assumed that all chicks get infected at the same time. To change the model from an average broiler to a whole flock of broilers with each broiler being diseased at a different moment, data are needed on temporal and spatial spread of parasites and hosts. Quantitative information on the uptake of oocysts from the litter is needed as well.

The current model is only valid for infection with *E. acervulina*. In practice, however, infection with more than one species occurs in about 40% of all flocks (Chapter 3). For modelling mixed infections, information is needed about competition effects on immunity formation (Hein, 1975) and of pathogenic effects (Hein, 1976).

In the current model, rate of body weight gain is directly affected by infection. Subsequently, feed intake is decreased, as a function of metabolic weight, body weight gain, and the ability to metabolize feed relative to uninfected animals. In reality, however, growth rate is not directly influenced by infection, but is affected through the decreased feed intake and less efficient nutrient utilization (Adams *et al.*, 1992). In the model it was assumed that the ability to metabolize feed was reduced by 10% at maximum by subclinical infection. Takhar & Farrel (1979a,b) showed that with a higher infection dose up to 40% reduction is possible. The phenomenon of compensatory growth (Voeten *et al.*, 1988a) was not modelled on actual knowledge of the underlying mechanisms. This mechanism of compensatory growth needs more attention. Both above-mentioned aspects, decreased feed intake during infection, and compensatory growth, might become more important, since poultry producers change feeding strategies from *ad libitum* to restricted feeding schemes.

Only direct effects of coccidiosis had an influence on the economic outcome (final body weight, feed conversion). Indirect effects, as more veterinary costs or longer time to slaughter which means less grow-outs a year, were not included. The economic impact of coccidiosis in The Netherlands based on production losses (Voeten *et al.*, 1988b) is mainly at the farm level; in other words, the income of farmers is affected. Controlling coccidiosis does not necessarily mean less economic damage. Benefits of alternative control strategies might be inferior to costs of these strategies. On the other hand, consumers safety and demands with respect to reducing the use of medicinal products also should be seen as a benefit, but these are difficult to quantify in economic terms. Another aspect in an economic evaluation is that expected benefits of a solution of the problem is often

expressed in terms of increased profit. However, on the long term, the agricultural producer does not benefit from it, because improved efficiency will drive prices downwards.

Immunity

Infections by *Eimerian* parasites result in a variety of immune responses including the induction of a species-specific protection against subsequent challenge (Rose, 1987). Innate immunity is responsible for the elimination of parasites during the early phase of primary infection, whereas acquired immunity to *Eimeria* is usually considered in the context of its effect on secondary infections. Immunity to challenge infection usually results in reduction of oocyst numbers and decrease in clinical signs, and is influenced by many factors associated with host and parasite. Both types of immunity to *Eimeria spp.* depend upon host factors such as age, sex and genetic background, that can significantly influence the effector function of the immune system (Lillehoj & Trout, 1994). The presence of other diseases and stress also might have a negative influence on the immune system (Siegel, 1995). The crucial difference between innate and acquired immunity is that acquired immunity is highly specific for a particular antigen. During and after primary infection, the innate immune system acts as a first line of defense. After 48 hours (with *E. tenella*) elements of the adaptive system also can become activated and it is very difficult to distinguish innate from adaptive system responses. Moreover, after reinfection the innate and adaptive system are hardly distinguishable (Vervelde, 1995). Vervelde (1995) stated that expression of intestinal immunity is complex and protection to natural infection with *Eimeria* spp. is not attributable to a single effector mechanism. In *E. acervulina* infections, protective immunity is induced by early parasite stages (from penetration up to early schizont formation) (Jenkins *et al.*, 1991). For *E. maxima* this is induced by the second generation of schizonts (Rose & Hesketh, 1976). Rose & Hesketh (1976) found sexual stages of *E. maxima* to be most susceptible for immune inhibition. Rothwell *et al.* (1995), however, found that in immune animals, the development of *E. maxima* administered in the challenge inoculum is halted shortly after invasion (Rothwell *et al.*, 1995).

So, immunity development against coccidiosis is very complex. Immunity status in the model was divided in several components: innate natural level of immunity (INNATE), maximum level of immunity attainable after exposure (MAXIMM), maximum development rate from innate to maximum immunity level (MAXGRO), time-lag between parasite contact and effect on immunity (TIMELAG), and cumulative number of damaged mucosa cells as a representation of the amount of antigen encountered and thus involved in immunity formation (CUMIMM). Simulation results were significantly influenced by MAXIMM, MAXGRO and TIMELAG.

Inclusion of a time-lag of 5 days was supported by experiments (Chapter 2.3 & 2.4).

Data are needed about INNATE, MAXGRO and MAXIMM. It is to be expected that these parameters are dependent on factors both of the host and the parasite. Factors of the host might include age, and genetic background. Lillehoj (1988) and Lillehoj *et al.* (1989) found genetic differences in immune responses to *E. tenella*. Also resistance to *E. acervulina* appears to be associated with MHC genes in broilers (Uni *et al.*, 1995). Lillehoj (1988) and Stiff & Bafundo (1993) found no effect of age, in contrast to Long & Millard (1979).

In the model it was assumed that every stage in the life cycle was equally effective in eliciting an immune response. Moreover, these stages are cumulated and above 20,000 stages the rate of attainment of maximum immunity becomes maximal. First, the number of 20,000 is an arbitrarily chosen number. In species of *Eimeria* it is common to have a life cycle of a number of distinct, sequential, asexual replicative generations, followed by a sexual phase with every different developmental stage having its own antigenic diversity as well as different capacity for inducing protective immunity (McDonald *et al.*, 1986; McDonald *et al.*, 1988; Tomley, 1994; Ovington *et al.*, 1995). Additionally, field strains and precocious lines of different *Eimeria* species exist with their own capacity to elicit protective immune responses (Shirley, 1989; Fitz-Coy, 1992; Lillehoj & Trout, 1993). Second, does a threshold exist in induction of protective immunity? Results shown in Chapter 2.4 do suggest some kind of influence of infective dose on rate of attainment of immunity. A possible phenomenon of a threshold may differ with age, since a higher oocyst production was found in older birds (Krassner, 1963), regardless of their genetic background (Lillehoj, 1988). So, more experimental and theoretical studies need to be done to study immunity formation dynamics in relation to the infective dose.

Anticoccidial efficacy

Anticoccidial efficacy in the model was assumed to be 75% between each stage in the life-cycle. Zero efficacy was not possible; the model crashed. In practice, however, complete resistance to anticoccidials is possible (Chapman, 1993a), indicating that the model has to be improved concerning this aspect. The allowed range for anticoccidial efficacy should be 0% to 100%. Moreover, different anticoccidials work against different stages of the life-cycle of *Eimeria* spp. In general, knowledge about anticoccidial drugs concerning practical applications is numerous, whereas knowledge about mode of action hardly is present (Chapman, 1994). In the current model, administration of different anticoccidials during grow-out is not possible. Thus, application of shuttle programmes with anticoccidials as used in practice cannot be simulated. It is necessary to adjust the model for this. Shuttle programmes make use of two anticoccidials each with their own efficacy. At a certain point during the grow-out, the first anticoccidial is replaced by a second one. Usually, the replacement time occurs somewhere after 1 to 3 weeks after start of the flock

cycle. By varying the efficacy of both anticoccidials (range 0 to 100%) simulations can be done for a shuttle programme, and their effects on immunity formation and economic outcomes can be tested.

Sometimes, immunity development occurs in birds given anticoccidial drugs, which do not absolutely suppress all stages in the parasitic life cycle (Chapman, 1994). Immunity stimulation in practice depends on frequency and intensity of exposure to oocysts in the environment (Chapman, 1978). However, use of anticoccidials and with that reduction of oocyst output in the environment might interfere with immunity development. It should be kept in mind that oocysts may have a decreased ability to sporulate when some anticoccidials are used (Norton & Joyner, 1978; Löwenstein & Kutzer, 1989). This has an influence on the number of infective oocysts in the environment, which subsequently influences infection pressure and protective immunity development.

The experiment described in Chapter 2.5 showed an interaction between immunocompetence of the host and level of anticoccidial drug application on immunity development. This was, however, only tested for one particular anticoccidial and at very low infection levels. More data are needed with respect to infection level and other types of anticoccidials.

External factors

In the model only the population dynamics of the parasite and its effects on production were described. Initially, external factors were left out of the model. Also, oocysts were assumed to be present at the start of each grow-out. This is not necessarily true. Therefore, in Chapter 3, the prevalence and risk of coccidiosis were determined in the field. Possible factors were identified and quantified with an existing database of a large number of commercial broiler farms. Now that risk factors are quantified, they provide knowledge on the relative impact, facilitating future implementation into the current model or other models (*e.g.* decision support models). Also, they could be used to set priorities for management, advisory and intervention programmes to reduce losses due to the disease. At present, new animal health and risk management strategies are being developed. Reducing or controlling animal diseases could be seen as a quality aspect of the production process. Current animal health management is product oriented and problems are only being solved after occurrence. Process oriented management, as in GXP (Good X Practice) codes and HACCP (Hazard Analysis of Critical Control Points) methodology will lead to control of the production process and improvement of product quality (*e.g.* broiler meat). For such management methods identification and quantification of risk of introduction, transmission and emission of pathogens within and between herds are needed (Noordhuizen & Welpelo, 1996). Risk factors (as in Chapter 3) can be used to determine critical control points in the

production process and hence to inform individual farmers about different animal, environment, and management related risk factors present on their farms and what preventive measures possibly can be taken.

In coccidiosis, the factors might have a direct effect on the population dynamics and therefore an indirect effect on the production of the host. However, external factors also may have a direct influence on production. An example of such factor is applied light regimen in broiler houses. Intermittent lighting improves production (Classen *et al.*, 1991). However, the risk on coccidiosis is increased with intermittent lighting (Henken *et al.*, 1992). This might be explained with discontinuous feed intake, and therefore discontinuous medication which leads to a complete loss of the anticoccidial activity as suggested by Long *et al.* (1983). Also, discontinuous feed intake and concomitant reduction in anticoccidial intake should be considered in evaluating control strategies, especially with existing feed restriction programmes.

The opposite also might occur; eliminating certain risk factors might decrease the risk on coccidiosis, but might increase risk on other diseases or might negatively influence production. Thus, before advising a farmer based on model outcomes, also the economic consequences have to be considered. Spread of coccidiosis between farms was not an aspect of this research, but might be important in designing a control strategy, focusing on integrated livestock production chains.

Summarising directions for further research

Much coccidiosis research is focused on immunity development processes, with an ultimate goal to find an effective vaccine. The dynamics of an infection with *Eimeria* spp. together with the dynamics of protective immunity development are important in this. The need for a better understanding of the interaction between infection and immune system for infections as occur in practice, was highlighted in this thesis. Effects of immunity formation and immunity formation in interaction with different types of anticoccidials need to be studied further. Especially, since feeding strategies change from *ad libitum* to restricted feeding and this might significantly influence efficacies of anticoccidial drugs. This changing attitude towards feeding strategies also has an impact on effects of infection on growth and possibilities for compensatory growth and needs further evaluation.

The interaction between infection, immunity and anticoccidial drugs also has consequences for spread of oocysts in the environment of chickens and needs to be considered. Spread of infection in a house, risk of introduction in a house or farm should be known to be helpful in risk management programmes. Also, economic aspects should be studied more profoundly.

PRACTICAL IMPLICATIONS

In the introduction of this chapter, it was mentioned that the project described in this thesis should contribute to a better decision basis for improving control strategies for coccidiosis. Some practical implications derived from results found in our research follow below.

Differences between number of oocysts sporulated in dry or wet litter were not obvious. So, with respect to coccidiosis, litter quality appears to be of minor importance. Litter quality, however, might reflect management skills of the farmer or indicate other problems as other diseases or nutritional malfunctioning in broiler flocks.

It was shown that the effect of a coccidiosis infection was more severe in chickens with a lowered immune response. Therefore, it is important to avoid stress and other diseases as these may negatively affect immune responses. Prevention of coccidiosis is also important regarding public health since it is known that coccidial infections can be predisposing for *Salmonella* infections and deteriorate and prolong the *Salmonella* shedding (Takimoto *et al.*, 1984; Qin *et al.*, 1995).

If an infection with *Eimeria* cannot be prevented, an intermediate initial contamination level is optimal in terms of revenues. This intermediate contamination level cannot be expressed in practical implications for cleaning and disinfection measures, but should be used in the design of vaccines. All the more because less cleaning and disinfection probably has its repercussion on other disease agents too. Additionally, it was found that the risk of having coccidiosis, with negative effects on production, was significantly higher, when the previous flock was infected. This was especially remarkable for *E. tenella* and *E. maxima*. Other risk factors found, were factors facilitating introduction of infection in the flock, or were related to hygienical measures. With help of the list of risk factors, the individual farmer can determine his possibilities for improving the control of coccidiosis. Generally, prevailing advices on sanitary measures remain valid.

CONCLUDING REMARKS

A model can never be more accurate than the data that are underlying the model. In our coccidiosis model still several assumptions exist which are not verified with experimental work. Quantitative comparison between model and experiments showed discrepancies, which illustrate the need for better calibration and change of relationships in the current model. Making a decision based on current model outcomes is only allowed if the person making the decision is totally aware of consequences and constraints, and

accounting for major unresolved assumptions. Therefore, concerning the fact that this coccidiosis model is still in its developing stage, concrete decisions cannot be made yet. Nevertheless, the value of the current simulation model is that it helps to increase understanding and insight in aspects which are really important and which are of minor importance. With this, the directions for further research priorities are identified, leading to more efficient use of research resources. Just this interaction between simulation and experimental and observational work is of crucial importance.

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Summary

Summary

INTRODUCTION

Coccidiosis is an infectious disease caused by protozoa of the species *Eimeria* and has negative effects on the growth and feed efficiency of commercially reared broilers. To prevent economic losses due to these negative effects on production, anticoccidial drugs are used continuously. Despite standard use of anticoccidial drugs in the chicken's diet, losses due to coccidiosis in intensively reared chickens are enormous. These losses are approximately 2.2% of slaughter value. Much research is focused on attempts to induce protective immunity and to understand mechanisms of it, and with that trying to develop an effective vaccine. Until now, the few available vaccines have proven to be only cost-effective in broiler breeder flocks, not in broiler production. Therefore, more information is needed for a way to control the negative effects of coccidiosis infection. Knowledge about factors influencing introduction, course and spread of coccidiosis is needed.

A research project was started to model *Eimeria acervulina* infections in broilers. The objective was to increase understanding of factors that influence the dynamics and mechanisms of an *Eimeria* infection in broilers and its effect on production. This was done with combined theoretical, experimental and field work. First, a model was developed. By performing experiments with the model it was determined which experiments had to be done with the system (*i.e. E. acervulina* infection in broilers) to validate principles and phenomena found in simulation results. The main objective of the research project described in this thesis was focused on (qualitative) validation of the simulation model.

SIMULATION MODEL

In Part I, the deterministic simulation model is presented. It describes the population dynamics of the parasite *Eimeria acervulina* (Chapter 1.1), and effects of the parasite on broiler production (Chapter 1.2). The model includes assumptions about development of immunity to *E. acervulina* infection and effects of application of anticoccidial drugs (Chapter 1.1). Effects of the parasite on broiler production characteristics were presented in Chapter 1.2. The model shows a phase of compensatory growth during which earlier negative effects are (partly) counterbalanced. The results show that achieved production characteristics during a flock cycle depends on the initial contamination level. Both, high and low initial contamination levels resulted in a lower average daily gain, a worse feed to gain ratio, and a reduced net revenue compared to an intermediate contamination level.

The effect on body weight gain, feed to gain ratio, and net revenue of changing parameters of the model was determined (Chapter 1.3). It was indicated that the model is relatively insensitive to values of the parameters sporulation rate, mortality rate and intake rate of oocysts. Results showed that the model is especially sensitive to parameters related to anticoccidial drug efficacy and host immunity. Following aspects of host immunity were most important: maximum attainable immunity, the rate at which maximum immunity is attained and time-lag between parasite contact and development of protective immunity.

QUALITATIVE VALIDATION

Most emphasis was placed on experimental validation of parameter estimates (Chapter 2.1) and hypotheses derived from simulation (Chapter 2.2 to 2.5).

An experiment was done to estimate parameters related to the sporulation process. Changing sporulation rate did not lead to large changes of simulation results. However, since experts in coccidiosis presumed climatic and litter conditions very important in facilitating infection with *Eimeria* spp., it was decided to study sporulation under circumstances encountered in practice. The results showed that sporulation percentage is not the most important factor, since results were almost equal for all tested environmental conditions. So, in both model and simulated practical conditions sporulation rate appeared to be of minor importance.

Validation was carried out for the model as a whole to obtain evidence supporting the hypothesis of existence of an optimal initial contamination level regarding broiler productivity (Chapter 2.2). The results showed that from a qualitative perspective the simulation model behaves realistic for those initial contamination levels tested. It was, thus, confirmed that an optimum infection level exists at which economic loss is lowest, assuming that oocysts are present in the broiler house. Quantitative agreement between simulation and experimental results was less satisfactory.

Two experiments were conducted to validate the model for parameters of the broilers' immunity status. In the first experiment (Chapter 2.3) it was validated that with a lowered immune responsiveness the effects of an *E. acervulina* infection on broiler production were disproportionally larger than in non-immunocompromised birds. Overall, the results of this experiment showed that the simulation model qualitatively agreed with experimental results. However, in the simulation model, effects of differences in general immune responsiveness appear to overestimate the effects as were observed in the experiment. A time-lag of 120 hours between establishment of infection and immunity development was assumed in the simulation model. Furthermore, it was assumed that this

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was dependent on infection level. Experiments (Chapter 2.3 & 2.4) supported inclusion of these aspects in the simulation model.

In the sensitivity analysis it was shown that with both a lowered immuno-competence of the host and a lowered anticoccidial efficacy, performance of broilers is disproportionally worse than in normal non-immunocompromised animals treated with an effective anticoccidial drug. This was tested experimentally (Chapter 2.5). The experimental results were in contrast with the hypothesis. The largest immunosuppression effect was found in groups with higher drug doses. This may have been caused by the very low infection level applied in this study, as well as by the immunosuppressive agent used. Clearly, this aspect of the model needs more attention.

In the simulation model it was assumed that infection with *E. acervulina* occurs in every flock. Data from poultry practice were analysed to investigate the actual prevalence and factors influencing the occurrence in a flock cycle (Chapter 3). In 63% of all flocks coccidiosis occurred. The results of the study showed an enhanced risk of coccidiosis due to environmental and management factors that increase the risk of introducing contamination or have a relation with hygienic measures.

DISCUSSION AND CONCLUSIONS

In the General Discussion the results are discussed with respect to the initial objective. Validation experiments showed qualitative agreement between experimental and simulation results, resulting in some confidence in the model as a whole. Nevertheless, a lot of work has to be done to adjust and improve the current model. Quantitative comparison between model and experiments showed discrepancies, which illustrate the need for better calibration and change of relationships in the current model. Decisions based on model outcomes are only allowed if the decision maker is aware of consequences and constraints, and accounting for major unresolved assumptions. Therefore, considering that the coccidiosis model is still in its developing stage, decisions cannot be made yet and further model-building activities should be coupled with animal experiments.

Samenvatting

Samenvatting

In dit hoofdstuk worden de achtergronden, doelstellingen en uitkomsten van mijn promotieonderzoek beschreven voor al die geïnteresseerden die niet thuis zijn in de wereld van pluimvee en coccidiose. Het is dus geen vertaling van de engelstalige "Summary", maar een populaire weergave.

In dit proefschrift staat een onderzoek beschreven naar de ziekte coccidiose, welke bij kippen vaak voorkomt. De ziekte wordt veroorzaakt door parasieten, *Eimeria* genoemd, die zich bevinden in de darmen van kippen. Het zijn eencelligen die alleen met behulp van een microscoop goed gezien kunnen worden. Bij kippen kunnen 9 soorten *Eimeria* voorkomen. *E. acervulina* komt wereldwijd het meest voor. Dit proefschrift gaat voornamelijk over deze laatste soort.
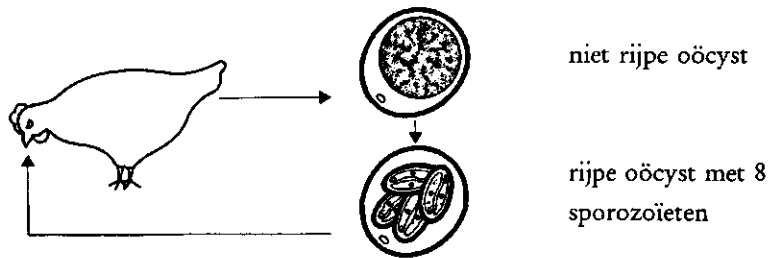
DE LEVENSCYCLUS VAN DE PARASIET

De levenscyclus van de parasiet vindt gedeeltelijk binnen de kip en gedeeltelijk buiten de kip plaats (Figuur 1). De kringloop begint als rijpe oöcysten (eitjes) van de parasiet worden opgenomen. In het maagdarmkanaal gaat de wand van de eitjes stuk en komt de schadelijke inhoud, de sporozoïeten, vrij. Deze sporozoïeten dringen de darmwand van de kip binnen en vermenigvuldigen zich daar zeer sterk door deling. Hierdoor wordt de darmwand ernstig beschadigd. Bij de laatste deling ontstaan mannelijke en vrouwelijke cellen die samensmelten tot nieuwe oöcysten. Deze verlaten de kip via de mest, vaak met miljoenen tegelijk. De tijd tussen opname en uitscheiding duurt afhankelijk van de soort *Eimeria* 4 tot 7 dagen. Zeer verse mest is niet besmettelijk. Eerst moeten de eitjes namelijk nog een proces van rijping doormaken, waarbij in de eitjes sporozoïeten worden gevormd. Voor deze rijping zijn zuurstof, warmte en vocht nodig. Het rijpingsproces duurt, afhankelijk van de soort, 1 tot 2 dagen. Over het algemeen zijn de omstandigheden op kippenbedrijven goed voor rijping van de eitjes.

DE GEVOLGEN VAN COCCIDIOSE

De kip zal door de darmwandbeschadiging slechter eten; het dier voelt zich immers niet zo lekker. Verder wordt het opgenomen voedsel slechter benut, omdat door de beschadiging minder voedingsstoffen doorgelaten kunnen worden. Door deze 2 factoren zal

een geïnfecteerde kip slechter groeien dan een niet geïnfecteerd dier. Voor de vleeskuikenhouderij betekent dit een groot financieel verlies. Kuikens worden vanaf het moment van uitkomen uit het ei (ca. 40 gram) zo snel mogelijk (in ca. 6 weken) grootgebracht tot het slachtgewicht (ca. 2000 gram). Iedere gram minder die naar de slager gaat, of iedere dag langer dat een dier in de stal moet blijven om het eindgewicht te bereiken levert verlies op. Er is berekend dat ieder dier wat coccidiose doormaakt ca. f0,06 verlies oplevert door de slechtere productieresultaten. Nu lijkt dit niet veel, maar voor een gemiddeld vleeskuikenbedrijf met 45.000 plaatsen en 7 mestrondes per jaar kan dit een schade van ca. f20.000,- betekenen.

niet rijpe oöcyst

rijpe oöcyst met 8 sporozoïeten

Figuur 1. Een schematische weergave van de levenscyclus van de parasiet

DE BESTRIJDING

Schoonmaken is belangrijk om infectie te voorkomen. Dit betekent wel dat alle ziekteverwekkers uit de stal verwijderd moeten worden, omdat ontsmetten bijna onmogelijk is: behalve zwavelzure ammoniak kan geen enkel middel de parasiet doden.

Om coccidiose te voorkomen worden aan kippen anticoccidiosemiddelen toegediend. Dit mag echter maar tot een aantal dagen voor het slachten, om eventuele resten van het medicijn in het kippenvlees te vermijden. Dit betekent dat de ziekteverwekker in deze periode de kans krijgt om alsnog schade aan te richten. Een ander probleem is dat, door het veelvuldige gebruik, de parasieten ongevoelig worden voor de huidige medicijnen. Er moet dus een andere oplossing gevonden worden om het coccidiose-probleem onder controle te houden. Vaccineren zou een oplossing kunnen zijn. Tot nu toe is er echter nog geen vaccin beschikbaar voor de commerciële vleeskuikenhouderij.

HET COMPUTERMODEL

Er wordt dus op verschillende manieren gezocht naar een methode om de infectie zo goed mogelijk onder controle te houden. Het zou makkelijk zijn om voorspellingen te kunnen doen over uitkomsten van verschillende bestrijdingsstrategieën. Hiervoor moet echter wel een goed inzicht bestaan in het verloop van de infectie en de factoren die hierbij belangrijk zijn. Om een beter inzicht te verkrijgen, is een computermodel ontwikkeld. In dit computermodel zijn de infectie en de relaties tussen de kip en de infectie beschreven.

Het model dat de infectie met de coccidiose-parasieten beschrijft (Hoofdstukken 1.1 & 1.2), bestaat uit wiskundige vergelijkingen. Het model start o.a. met het aangeven van het infectieniveau, namelijk hoeveel oöcysten er aanwezig zijn aan het begin van een mestronde. Het model gaat dan rekenen en laat o.a. zien wat het eindgewicht is in vergelijking met niet geïnfecteerde dieren en hoeveel voer nodig is geweest om dat gewicht te bereiken. Door waardes van factoren, waaruit de wiskundige vergelijkingen zijn opgebouwd, te veranderen en het model opnieuw te laten rekenen, wordt duidelijk of de uitkomst van het model heel erg verandert (Hoofdstuk 1.3). Wanneer dit factoren zijn waarvan nog weinig bekend is, is dit een reden om er meer onderzoek aan te doen. Een andere reden om meer onderzoek te doen is dat een uitkomst juist niet veel verandert bij verandering van een parameter, terwijl dat volgens experts wel zou moeten gebeuren. In experimenten wordt dan bekeken of datgene wat uit de "computerkippen" komt, overeenstemt met dat wat er in echte kippen gebeurt. Dit proces wordt valideren genoemd en vormde het grootste gedeelte van mijn promotieonderzoek.

DE EXPERIMENTEN

Uit het computermodel kwam o.a. naar voren dat voor het verloop van de infectie en de uiteindelijke groei en voederconversie (=hoeveel voer benodigd voor 1 kg groei) het aantal oöcysten wat rijp wordt in de omgeving van de kippen niet belangrijk is. Volgens experts klopt deze uitkomst niet. Zij denken dat de mate van rijping erg belangrijk is en dat dit sterk afhankelijk is van temperatuur, luchtvochtigheid en kwaliteit van het strooisel waarop de kippen leven. Dit is getoetst in een experiment. Het bleek dat het aantal rijpe oöcysten onder allerlei omstandigheden ongeveer hetzelfde was. Dus zowel in het experiment als het model was de mate van rijping minder belangrijk (Hoofdstuk 2.1).

Een opvallende uitkomst uit het model was dat het verlies per kuiken het hoogst was bij lage of hoge besmettingen aan het begin van een mestronde. Een tussenliggende dosis is optimaal; het verlies is daar het minst, aannemende dat er altijd oöcysten in de stal

aanwezig zijn. Dit fenomeen is in een experiment bekeken. De resultaten toonden aan dat het fenomeen van een optimale besmetting ook in werkelijkheid geldt (Hoofdstuk 2.2).

De mate waarin een kip weerstand kan bieden tegen een ziekteverwekker kwam als een erg belangrijk onderdeel van het model naar voren, evenals de mate van werkzaamheid van het anticoccidiosemiddel en de wisselwerking tussen deze twee factoren. Drie experiment zijn gedaan om deze factoren of onderdelen van deze factoren te toetsen (Hoofdstuk 2.3 t/m 2.5). De meeste proeven met coccidiose worden gedaan met normaal gezonde dieren. In de praktijk kunnen echter allerlei andere ziektes aanwezig zijn, waardoor de weerstand van de dieren minder is. Ook kunnen dieren door allerlei omstandigheden gestressed zijn, wat ook kan resulteren in een verminderde weerstand. Uit het computer-model werd afgeleid dat het effect van een coccidiose-infectie op de groei onevenredig groter was wanneer de weerstand ook maar iets verminderd was. Om dit te kunnen meten, is aan het voer van kippen een stof toegevoegd die de weerstand vermindert. Vervolgens zijn de dieren geïnfecteerd en gedurende een aantal weken gevolgd en vergeleken met geïnfecteerde dieren die gewoon voer kregen, dus zonder de weerstandsverlagende stof. In de verschillende experimenten zijn respectievelijk de dosis van de weerstandsverlagende stof gevarieerd, de hoogte van de besmettingsdosis, en de hoeveelheid anticoccidiosemiddel in het voer. Uit deze 3 experimenten bleek dat sommige aannames in het computermodel klopten en anderen aangepast moeten worden.

Een aanname in het computermodel was dat oöcysten altijd aanwezig zijn in een kippenhok. Om deze aanname te onderzoeken zijn gegevens bekeken van 4774 mestrondes op 177 vleeskuikenbedrijven. Deze gegevens zijn gedurende een aantal jaren verzameld door een dierenartsenpraktijk. Het bleek dat in 63% van alle mestrondes coccidiose voorkwam. In het onderzoek was verder bekeken welke factoren op een bedrijf of de omgeving van het bedrijf een risico zijn voor het optreden van de infectie. Een opvallende uitkomst was dat het risico op coccidiose, afhankelijk van de soort, 2 tot 11 keer zo groot was, wanneer in de vorige mestronde in hetzelfde hok ook coccidiose was opgetreden, vergeleken met de situatie waarin in de vorige mestronde geen coccidiose gevonden kon worden.

TOT SLOT

Na 4 jaar onderzoek is duidelijk geworden dat er nog veel aan het computermodel moet gebeuren om het te kunnen gebruiken om verschillende bestrijdingsstrategieën te vergelijken.

Curriculum vitae

Elisabeth Anna Maria Graat werd op 2 juli 1965 geboren te Wanroy. In 1983 behaalde zij het V.W.O. diploma aan het Mgr. Zwijssen College te Veghel. In hetzelfde jaar werd begonnen met de studie Zoötechniek, oriëntatie Veehouderij, aan de toenmalige Landbouwhogeschool te Wageningen. In september 1989 studeerde zij af, met als afstudeervakken Gezondheids- en Ziekteleer en Pluimveeteelt. Na haar afstuderen is ze voor een maand werkzaam geweest als practicumassistent bij de Landbouwuniversiteit. Vervolgens is ze een jaar projectleider geweest bij het Nederlands Varkensstamboek voor uitvoering van onderzoek t.b.v. selectiemesterijbiggen. Hierna heeft ze als toegevoegd docent "epidemiologie" op de Vakgroep Veehouderij van de Landbouwuniversiteit een nieuw onderwijselement mee helpen opzetten. Vanaf 1 april 1992 tot 1 juli 1996 was ze werkzaam als Assistent In Opleiding bij de Vakgroep Veehouderij van de Landbouw-universiteit en verrichtte het in dit proefschrift beschreven onderzoek. Per 1 juli 1996 volgde de aanstelling als universitair docent "epidemiologie" bij de sectie Gezondheidsleer-en Reproductie van de Vakgroep Veehouderij.