A kinetic model for *Ascaridia galli* populations in chickens treated with mixed salts of copper and zinc

M. Gabrashanska[1], S. Teodorova[2]*, M. Galvez-Morros[3] and O. Garcia Martinez[4]

[1]Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev str., bl 25, 1113 Sofia, Bulgaria: [2]Institute for Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences, 72 Tzarigradsko shaussee, 1784 Sofia, Bulgaria: [3]Departamento de Patologia, Facultad de Veterinaria, Universidad Complutense, Carreterade Puerto de Hierro s/n, 28040 Madrid, Spain: [4]Instituto de Ciencia de Materiales, Serrano, 113 CSIC, 28006 Madrid, Spain

Abstract

The action of mixed salts of copper and zinc (basic and neutral) on Hisex chickens experimentally infected with *Ascaridia galli* has been studied. The data show that the lowest host mortality and decrease in body weight gain and the highest reduction in nematode loading occurs in infected chickens treated with basic salts (in comparison with infected chickens, untreated or treated with neutral salts). A mathematical model has been proposed to provide a quantitative interpretation of the observed results. The model solutions of the kinetics of parasite numbers and of the gain in body weight are in a good agreement with the experimental data. One of the kinetic parameters in the model is defined as a phenomenological constant of the host immune response. Its value is determined in the case of infected and untreated chickens.

Introduction

Ascaridia galli infections have been shown to lead to an imbalance of some trace elements (zinc, cobalt, copper, iron) in chickens (Gabrashanska *et al*., 1986). Experimental ascaridiosis has resulted in a decrease in body weight gain, high host mortality and other secondary pathological symptoms. In the present paper we discuss the action of mixed neutral and basic salts on chickens infected with *A. galli*. A quantitative interpretation of the results has been proposed.

In poultry, copper and zinc nutrients are essential for optimal growth performance (Davis & Mertz, 1987). The ordinary corn–soybean diet in poultry is frequently supplemented with an excess of copper and zinc in an effort to meet or exceed their nutritional requirements. Up to now, only neutral salts of copper and zinc ($ZnSO_4$, $CuSO_4{:}5H_2O$) are generally used to correct mineral deficiencies and they have been used in particular to restore the normal mineral balance of infected hosts. Gabrashanska *et al*. (1993) and Galvez-Morros *et al*. (1995) have shown that pure basic salts of zinc and copper can be used to correct losses in body weight, host mortality and worm burdens in chickens experimentally infected with *A. galli*. They can also replace neutral copper and zinc salts as therapeutic agents in ascaridiosis. Gabrashanska (1993) and Gabrashanska & Timanova (1993) demonstrated that pure basic salts of copper ($CuCO_3$, $Cu(OH)_2.nH_2O$ and $Cu_2(OH)_3Cl$) and zinc ($Zn_5(OH)_8Cl_2.H_2O$) have slight toxic effects on infected chickens with *A. galli*, but these salts (especially the copper basic salts) have antiparasitic actions. At the same

*Author for correspondence. Fax: +359 2 975 3619.

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time, basic salts stimulate the growth and development of the host. Mixed crystals of basic salts of copper and zinc ($Zn_{5-x}Cu_x(OH)_8Cl_2.H_2O$) have been synthesized for research purposes in our laboratory in Madrid and these were used in the first place in chickens with experimental ascaridiosis (Gabrashanska & Timanova, 1995).

A mathematical model is developed in the present study of the establishment of *A. galli* populations in untreated chickens or those treated with copper and zinc mixed salts (neutral and basic) and of the body weight gain of healthy and infected hosts. On the basis of the model, some kinetic parameters which characterize the phenomenological structure of the parasite–host system have been determined and these include the reduction rate constant of the parasite population and the relative rate of gain in body weight.

Materials and methods

One hundred and twenty male Hisex chickens (interlinear crossbreeding of Dutch Leghorn) were divided into four groups of 30 as follows: Group 0, control (non-infected and untreated); Group 1, infected with *A. galli* and untreated; Group 2, infected with *A. galli* and treated with a mixture of pure neutral salts of copper and zinc ($CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$); and Group 3, infected with *A. galli* and treated with mixed zinc-copper basic salts ($Zn_{5-x}Cu_x(OH)_8Cl_2.H_2O$) (zinc 49.4% and copper 11%).

The chickens from groups 1, 2 and 3 were infected orally with 450 embryonated *A. galli* eggs at 14 days of age. Five daily doses of mixed salts (neutral and basic, respectively) were given orally to each chicken in groups 2 and 3. Each dose comprised 6 mg copper and 8 mg zinc either in the form of neutral and basic salts.

Experimental and control chickens were similarly fed and maintained for up to 75 days post infection (p.i.). The basic diet consisted of a conventional corn–soybean meal diet with adequate amounts of vitamins and minerals, but with no zinc and copper supplementation (National Research Council, 1984).

The determination of parasite numbers commenced on day 10 p.i. covering a 75-day period. Measurements of host body weight, mortality and worm burden were made on days 10, 30, 45, 60 and 75 p.i., following a complete autopsy of the alimentary tract.

All data were checked for variance homogeneity using the statistical program 'Statigraphics 5.0'.

Mixed basic salts of copper and zinc: $Zn_{5-x}Cu_x(OH)_8Cl_2.H_2O$ (zinc 49.4%) and copper 11%) were prepared by hydrolysis of $ZnCl_2$ and 0.2 M $CuCl_2$ with NaOH 0.4 M at 60° for 24 h at the Laboratory of the Instituto de Ciencia de Materiales, CSIC, Madrid. The precipitate was filtered, washed with distilled water, ethanol and acetone, dried in a vacuum and maintained over phosphorus pentoxide. The prepared salts were characterized by elemental analysis, infrared spectroscopy, electron microscopy and X-ray diffraction (Cano Ruiz *et al.*, 1960).

Mathematical model

The host's infected organ (the intestine) is viewed as a unique 'cultivator' and it is assumed that the therapeutic agent (the mixed salts) is supplied evenly and constantly into it. The salts are exerted from the 'cultivator' at a rate proportional to their concentration. The rate of flow is accepted as being equal to the average rate of the passage in the chicken's alimentary tract. It is assumed that the parasites (i.e. the nematodes, *A. galli*) and the therapeutic agent are distributed evenly and from a homogeneous mixture and this allows the writing of ordinary differential equations.

The following differential equation is proposed for describing the growth of healthy uninfected chickens:

$$\frac{dP}{dt} = \mu$$

where P is the weight of the chicken and μ ($[\mu] = [\mathrm{g\,day^{-1}}]$) is the relative rate of gain in body weight.

The weight of healthy chickens increases linearly, which is in good agreement with the data provided by the Dutch Company Euribrigh regarding the development of hybrid Leghorn birds (Hisex white hybrid, Euribrigh-Holland) during the first 20 weeks after hatching. A nutritional substrate is not regarded as a limiting factor.

During their development the nematodes disturb the nutrient digestibility of the host, which in turn reduces its growth rate. The nematodes reduce to a certain degree the nutritional reserves of the host and at the same time the nematode toxins adversely influence the enzyme systems in the intestinal mucosa and interfere with the normal absorptive processes (Akert, 1942; Vassilev *et al.*, 1973). The decrease in gain in body weight in chickens infected with *A. galli* is assumed to be proportional to the number of nematodes:

$$\frac{dP}{dt} = \mu - kN$$

Following infection the number of larvae and adult nematodes decreases as a result of an immune response by the host. This process may be described in the infected untreated chickens (Group 1) with the differential equation:

$$\frac{dN}{dt} = -\nu N$$

where N is the worm number in the host intestine, dN/dt is the rate of reduction of the worm population and ν ($[\nu] = [\mathrm{day^{-1}}]$) is the relative reduction rate constant.

As host immunity results in a reduction in worm burden, ν is considered as an integral characteristic of the host immune status and ν is defined as a phenomenological constant of the host immune response, i.e. the immunological constant.

Worm reduction in chickens from groups 2 and 3, which are treated with neutral and basic salts respectively, is more dependent on the anti-nematode action of the salts.

The kinetics of the establishment of nematode populations in the intestine of the chickens and of the gain in body weight with or without salt treatment can be presented by the following system of ordinary non-linear

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differential equations:

$$\frac{dS}{dt} = \frac{\phi S_o^2 - \beta j S^2}{2So} \qquad j = 2,3 \qquad (1)$$

a) $$\frac{dN}{dt} = -\nu N$$

b) $$\frac{dN}{dt} = -\nu N - aSN \qquad (2)$$

c) $$\frac{dN}{dt} = -\nu_B N - aSN$$

a) $$\frac{dP}{dt} = \mu$$

b) $$\frac{dP}{dt} = \mu - k_i N + b\mu_s \qquad i = 1,2,3 \qquad (3)$$

under initial conditions:

$$t_o = 0,\ S(t_o) = 0,\ N(t_o) = N_o,\ P(t_o) = P_o \qquad (4)$$

where t_o is the time moment corresponding to 24 h p.i. We postulate $t_o = 0$. S is the quantity of the therapeutic agent (the quantity of salts) in the biomass of nematodes at a given time t, S_o is the salt quantity entering the host's alimentary tract (the average daily dose of zinc and copper in the form of neutral or basic mixed salts given orally to each chicken), $\phi([\phi] = [\text{day}^{-1}])$ is the flow rate or dilution rate constant, determined as an average flow of passage through the alimentary tract divided by the average daily dose of salts), $\beta_j = \phi + \alpha_j$, where α_j is the rate constant of the resorbtion of the salts in the host's intestine (the resorbtion constant). α_2 (resp. β_2) corresponds to neutral salts and $\alpha_3(\beta_3)$ to basic salts. $a([a] = \text{day}^{-1}])$ is a rate constant of decreasing *A. galli* populations caused by the antiparasitic action of neutral or basic salts, i.e. the antiparasitic constant. We assume that a has the same value for both neutral and basic salts. ν_B is the constant for the immune response of chickens treated with basic salts (Group 3). The stimulating effect of the basic salts on the host is taken into account with the value of the constant ν_B. This stimulating effect expresses itself as a stronger immune response: $\nu_B > \nu$. The parameters k_1, k_2 and k_3 ($[k_i = [\text{g day}^{-1}]$) respectively for Groups 1, 2 and 3 are the relative rate constants of a decrease in body weight gain caused by the nematode infection, i.e. the retardation constants. The parameter μ_s is introduced as a relative rate of body weight gain resulting from cell stimulating processes. The binary variable b has the form:

$$\frac{\text{sign}(t - T_s) + 1}{2} = \begin{matrix} 0 \text{ at } t \le T_s \\ 1 \text{ at } t > T_s \end{matrix} \qquad (5)$$

T_s is the moment, in which the stimulating processes are visible.

The equations (2a), (2b) and (2c) describe the establishment of the nematode population in experimental Groups 1, 2 and 3 respectively. The equation (3a) presents the gain in host body weight for the control group (Group 0) and (3b) for other groups.

For the system of equations (1), (2) and (3) under the conditions (4) we obtain analytical solutions in the form:

$$S = S_o\sqrt{\frac{\phi}{\beta_j}}\frac{1 - e^{-\sqrt{\phi\beta_j}t}}{1 + e^{-\sqrt{\phi\beta_j}t}} \qquad j = 2,3 \qquad (6)$$

a) $N = N_o e^{-\nu t}$

b) $$N = N_o 2^{2\theta}\frac{e^{-(\nu+\theta)t}}{(1 + e^{-\sqrt{\phi\beta_2}t})^{2\theta}} \qquad (7)$$

c) $$N = N_o 2^{2\theta}\frac{e^{-(\nu_B+\theta)t}}{(1 + e^{-\sqrt{\phi\beta_3}t,})^{2\theta}}$$

a) $P = P_o + \mu t$

b) $$P = P_o + (\mu + b\mu_s)t - \frac{k_1 N_o}{\nu}(1 - e^{-\nu t})$$

c) $$P = P_o + (\mu + b\mu_s)t - \frac{k_2 N_o 2^{2\theta}}{\nu + \theta}(1 - e^{-(\nu+\theta)t}) \qquad (8)$$

d) $$P = P_o + (\mu + b\mu_s)t - \frac{k_3 N_o 2^{2\theta}}{\nu_B + \theta}(1 - e^{-(\nu_B+\theta)t})$$

where $\theta_j = aS_o\sqrt{\frac{\phi}{\beta_j}}$.

The solutions are: (8a) for Group 0; (7a) and (8b) for Group 1; (7b) and (8c) for Group 2; (7c) and (8d) for Group 3.

The immunological constant ν may be determined from the equation (7a) after taking in a logarithm:

$$\ln N = \ln N_o - \nu t \qquad (9)$$

This is an equation of a straight line with an angular coefficient ν. Using the values of N, determined by the experiment, we can construct a plot of $\ln N$ as a function of time.

Results and Discussion

The mortality of experimental chickens is presented in fig. 1, indicating that survival is lowest in untreated chickens (Group 1). The majority of chickens died up to day 30 p.i. There is a significant difference between Groups 2 and 3 with respect to the survival of the birds: those treated with basic salts show a lower mortality compared with chickens treated with neutral salts. This may be due to both the stimulating and antiparasitic effects of mixed crystals of the basic salts. Previous studies have shown that zinc stimulates the immunity of chickens and that copper has an antiparasitic action (Babenko & Reshetkina, 1971; Stepanjan & Sakyljan, 1981). The utilization of basic salts of copper and zinc by the host organism is lower compared with neutral salts and therefore their action is prolonged with lower toxicity (Gabrashanska & Timanova, 1995).

The model solutions (6) for the therapeutic agents (mixed neutral and basic salts respectively) are presented in fig. 2. We take into account the lower utilization of basic salts with the value of the parameter α_j : α_3 is smaller than $\alpha_2(\alpha_2 > \alpha_3)$, i.e. the resorbtion of basic salts in the alimentary tract is smaller, resulting in a higher concentration of basic salts in the intestine of the chicken. The dilution rate constant ϕ was determined, considering that

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the time required for passage of salts along the intestinal tract is 4 h and the length of the tract is 1.00–1.50 m (Bell & Freeman, 1971).

Now some comments about the equation (1) are presented. dS/dt is the total change of the quantity S with time. dS/dt increases with the salt flow $\phi S_o/2$ entering the host's alimentary tract and decreases with the flow $[(\phi + \alpha_j)/2](S^2/S_o)$ which is exerted from the tract. The exerting salt flow is proportional to the square of the salt concentration in the alimentary tract at a given moment t,

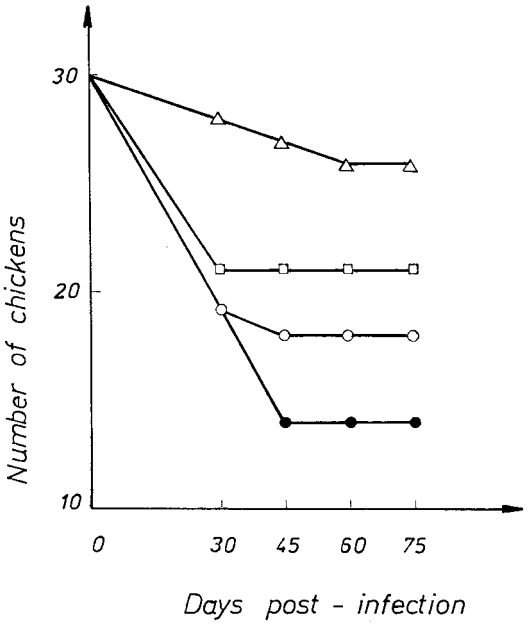

Fig. 1. The survival of chickens experimentally infected with *Ascaridia galli* up to day 75 post-infection. △, control group; ●, Group 1 – infected untreated chickens; ○, Group 2 – infected chickens treated with neutral salts; □, Group 3 – infected chickens treated with basic salts.

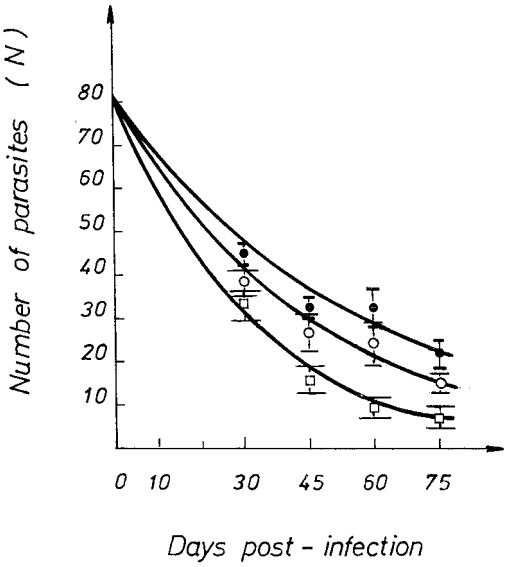

Fig. 3. The establishment of *Ascaridia galli* in the alimentary tract of three groups of Hisex chickens (theoretical curves and experimental points). ●, Group 1 – infected untreated chickens; ○, Group 2 – infected chickens treated with neutral salts; □, Group 3 – infected chickens treated with basic salts.

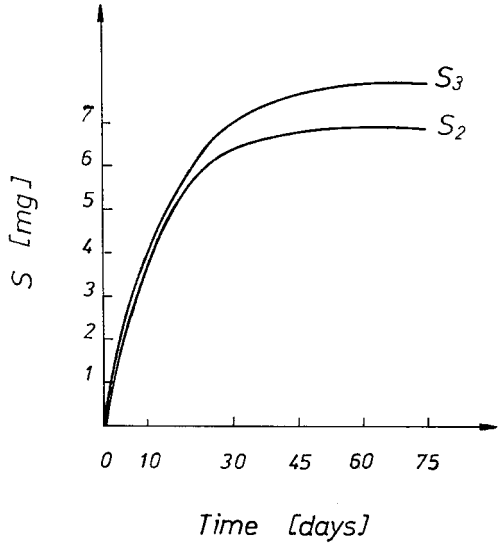

Fig. 2. Relationship between the biomass of *Ascaridia galli* occupying the alimentary tract of the chickens and the quantity of mixed salts during the experimental period (model solutions). S_2, neutral salts; S_3, basic salts.

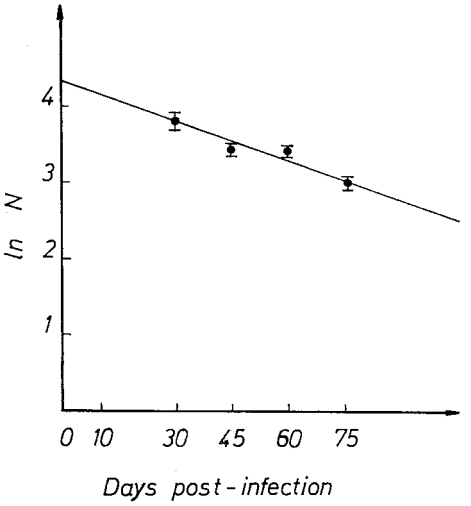

Fig. 4. Determination of the phenomenological constant of the host immune response ν on the basis of equation (9) using experimental measures of *Ascaridia galli* numbers in chicken Group 1 – infected untreated chickens.

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divided by S_o. Such a form of dependence (the square of the concentration) is justified by the good agreement of the solutions (6) of equation (1) with the experimental data relating to the establishment of *A. galli* in the host's alimentary tract.

Figure 3 demonstrates the change with time of *A. galli* populations in the host. Our statistical analysis shows p values in the interval: $0.01 = p = 0.05$. In all cases, the number of nematodes decreases and this is likely to be due to the effect of immune and allergic reactions of the host leading to the elimination of some of the helminth parasites (Bykoryukov & Tachistov, 1965). Lower numbers of *A. galli* were observed in Groups 2 and 3 and this is linked with the antiparasitic action of salts and with the stronger immune response of the host stimulated by the basic mixed salts. The reduction rate of worm numbers is highest in chickens treated with basic salts (Group 3). These results are in a good agreement with the theoretical solutions (7a), (7b) and (7c) and supports our concept that the nematode population in the host intestine decreases exponentially. The phenomenological constant of immune response ν was calculated on the basis of the experimental data. According to equation (9) ν is the angular coefficient of the straight line (fig. 4). We obtain:

$$\nu = 0.0167 \quad \text{day}^{-1} \tag{10}$$

The values of the resorbtion constants α_2 and α_3, antinematode constant a, retardation constants k_1, k_2 and k_3 and stimulation constant μ_s were calculated using the program 'Minuit'.

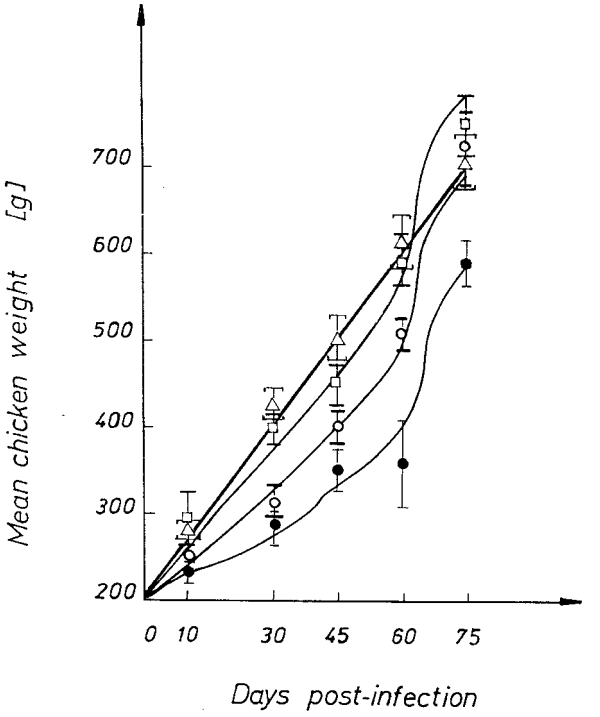

Fig. 5. Time course of the mean body weight in healthy chickens or infected with *Ascaridia galli* up to 75 days p.i. (model solutions and experimental points). △, control group; ●, Group 1 – infected untreated chicksn; ○, Group 2 – infected chickens treated with neutral salts; □, Group 3 – infected chickens treated with basic salts.

A good correlation was observed between host survival and changes in body weight gain (fig. 5). The growth of infected and untreated chickens (Group 1) is considerably reduced in comparison with that of healthy chickens. This retardation is most evident up to day 30 p.i. coinciding with optimum clinical symptoms in chickens with pathomorphological changes in the intestine (Vassilev *et al*., 1973). The retardation in growth in chickens treated with basic mixed salts is lowest. The model appropriately explains the experimentally observed time courses of body weight gain in the chickens of Groups 0, 1, 2 and 3. We have calculated the value of the relative rate of the control chickens' weight gain μ according to solution (8a), on the basis of the experimental data:

$$\mu = 6.5 \text{ g day}^{-1} \tag{11}$$

With reference to the parameter μ_s, the stimulating effect of the basic salts is not considered. It is well known that there is a wide range of stimulatory processes, which develop after some unfavourable influences on living organisms. Then the organism enters a characteristic state with a higher metabolism and physiological activity (Popoff, 1931). In general, these effects are related to an increase in enzyme activity and protein synthesis. Our analyses relate to phenomenological aspects and we cannot comment further about mechanisms at the cellular or molecular levels. But we introduce as a phenomenological parameter a stimulation weight gain rate μ_s and assume a linear dependence. Our theoretical results – solutions (8b), (8c) and (8d) are in a good agreement with the experimental data (fig. 5). By 60 days p.i., an accelerated weight gain in the chickens is observed and this is the period when the host is already in a condition of active rehabilitation.

The theoretical curves in the figs 2, 3 and 5 have been calculated using the following initial conditions and parameter values: $S_o = 10$ mg, $N_o = 80$, $P_o = 216$ g, $\phi = 0.08$ day^{-1}, $\alpha_2 = 0.09$ day^{-1}, $\alpha_3 = 0.05$ day^{-1}, $\nu_B = 0.0263$ day^{-1}, $a = 9.79.10^{-4}$ day^{-1}, $k_1 = 0.068$ g day^{-1}, $k_2 = 0.043$ g day^{-1}, $k_3 = 0.017$ g day^{-1}, $\mu_s = 1.6$ g day^{-1} and using (10) and (11).

In conclusion, this paper shows the visible effect of neutral and especially basic mixed salts in the treatment of infected chickens with *A. galli*. The basic salts with their double effect should be considered as potential pharmacological agents for ascaridiosis. The mathematical model allows us to formulate quantitatively some regulation of the establishment of *A. galli* populations under the conditions of the host immune response and also to explain changes in the body weight gain of hosts treated or untreated with neutral and basic salts. The kinetic parameter ν ('immunological constant') could be accepted as a characteristic phenomenological parameter in host–parasite systems and its value might be determined more precisely. It would be interesting to attempt to express ν as a function of some innate physiological parameter of the host and also of some characteristics of the helminth parasites (for example, intensity of infection, toxicity of the helminths). Our model is valid in cases where the

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infection dose is not very high and host age is characterized by an approximate linear time course of gain in body weight. The model also provides a basis for determining regimes for anthelminthic treatment with optimum effect.

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