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Effects of glycine–metal compounds on *Ascaridia galli*-infected chickens expressed by a kinetic model

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Abstract

The biogenic elements zinc, manganese and cobalt are essential for metabolic processes in animals. Compounds of nGly.Me^{2+}A. mH_2O (Me^{2+} = Zn^{2+}, Mn^{2+}, Co^{2+}; A = Cl^-, SO_4^{2-}, n = 1, 2; m = 2, 5), as supplements in the diet, were used separately on different experimental groups of male Hisex chickens to correct the mineral deficiency caused by *Ascaridia galli* infections. An amelioration of body weight gain, reduction of mortality and restoration of trace element levels were estimated in infected chickens. A mathematical model has been proposed for *A. galli* population kinetics in chickens, taking into account the stimulating effect of these elements on the nematodes. The model parameters are considered as phenomenological constants of the host–parasite system. An agreement with experimental data is observed using, for the parameters ψ, α, μ and μ_s, values equal to those calculated in previously investigated *A. galli*–chicken systems. For parameter ν (immunological constant) the same value was obtained as in a previous experiment with high infection. This model is likely to be suitable for a range of host–nematode systems, including varying degrees of infection and treatment with different trace elements.

Introduction

Mineral substances are important for correcting metabolic disturbances caused by helminthiases. Therefore, the role of trace elements in the pathobiochemistry of ascaridiasis needs more profound elucidation. Ascaridiasis is a widespread helminthiasis in birds and leads to slow growth, deformation of the skeleton, changes in reproductive function and other secondary pathological symptoms. *Ascaridia galli* infection also influences the balance of some trace elements such as zinc, copper, cobalt and manganese in the bird host (Gabrashanska *et al.*, 1987).

Neutral salts are often used not only as therapeutic agents to correct mineral deficiences but also to restore the normal mineral balance in infected hosts (Kincaid *et al.*, 1976; Southern & Baker, 1978; Balayan, 1982). Chelated or complexed trace elements with protein or single amino acids result in similar effects compared with elements from inorganic sources (Kratzer & Vohra, 1986; Flachowsky, 1997) with emphasis being focused on zinc–methionine (Wedekind & Baker, 1990), copper–lysine (Baker *et al.*,

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1991; Aoyagi & Baker, 1993), zinc–lysine (Aoyagi & Baker, 1993) and manganese–glycine (Gabrashanska *et al.*, 1999b).

The supplementation of animal diets in the form of complex metal compounds have shown satisfactory results in the treatment of parasites. Manganese–glycine and cobalt–glycine compounds have been used in chickens uninfected and infected with *A. galli* (Gabrashanska *et al.*, 1999b, 2001). These complexes can be used as alternative sources of trace elements in helminthiases. A mathematical model was developed for *A. galli* populations and the gain in body weight of birds infected with *A. galli* and treated with cobalt–glycine compounds (2Gly.$CoCl_2$.2H_2O; Gly.$CoSO_4$.5H_2O) (Gabrashanska *et al.*, 2002a).

In the present study, the effects of glycine–metal (Zn, Mn, Co) compounds, used on chickens infected with the nematode *A. galli*, have been estimated and compared. Body weight gain, mortality, parasite burden together with zinc, manganese and cobalt contents in chicken liver are considered as criteria for the bioavailability of these compounds in chickens. A mathematical model is used for the establishment of *A. galli* populations in untreated chickens or those treated with glycine–metal compounds and also for the gain in body weight in control and infected hosts. Some kinetic parameters characterizing the phenomenological structure of the *A. galli*–chicken system (the reduction rate of the nematode population and the relative rate of gain in body weight of the host), have been determined.

Materials and methods

The systems Gly-Me^{2+} A-H_2O have been investigated and the compounds nGly.Me^{2+}A.mH_2O ($Me^{2+} = Zn^{2+}$, Mn^{2+}, Co^{2+}; $A = Cl^-$, SO_4^{2-}, $n = 1, 2$; $m = 2, 5$) have been synthesized (Balarew *et al.*, 1994; Gabrashanska *et al.*, 2002b) to supply chickens with the biogenic elements Zn, Mn, Co and also with the amino acid L-glycine.

One-day-old male chickens belonging to the Hisex breed (a crossbreed of the Dutch Leghorn), were divided into eight groups of 30 as follows: group 0, control (uninfected and untreated); group 1, infected with *A. galli* and untreated and groups 2–7, infected and treated with the following compounds: group 2 with Gly.$ZnCl_2$.2H_2O; group 3 with Gly.$ZnSO_4$.5H_2O; group 4 with 2Gly.$CoCl_2$.2H_2O; group 5 with Gly.$CoSO_4$.5H_2O; group 6 with 2Gly.$MnCl_2$.2H_2O; group 7 with Gly.$MnSO_4$.5H_2O.

Control and experimental chickens were reared in a vivarium, placed on pine shavings in 1.2×3.6 m pens and maintained on a 24 h constant light schedule in heated (35°C), thermostatically controlled, stainless steel starter batteries with raised wire floors. Feeders and water containers were also of stainless steel construction to minimize environmental zinc, manganese and cobalt contamination. All chickens were fed on a conventional corn–soybean meal diet, formulated to meet the nutrient requirements of growing chickens (US National Research Council, 1994). The diet was Zn^{2+}, Mn^{2+} and Co^{2+} free and chickens were allowed access to food and water *ad libitum*.

Each chicken from groups 1–7 was orally infected by pipette with 1450 embryonated *A. galli* eggs as described by Permin *et al.* (1997) on day 14 post-hatching. Metal compounds were given in the food, commencing 7 days post infection (p.i.). Treatment was carried out acording to the following scheme: compounds were received for 5 days, followed by an interval of 7 days, then received for 5 more days, followed by another 7 day interval, and so on to the end of the experiment, covering a period of 75 days post-infection. Chickens received the elements Zn, Mn and Co, either in the form of chlorides or sulphates, in equal doses: for groups 2 and 3–0.12 g Zn^{2+} kg^{-1} food and 0.27 g or 0.14 g Gly kg^{-1} food; for groups 4 and 5–0.06 g Co^{2+} kg^{-1} food and 0.15 g or 0.08 g Gly kg^{-1} food; for groups 6 and 7–0.9 g Mn^{2+} kg^{-1} food and 2.5 g or 1.23 g Gly kg^{-1} food.

Worm burdens and chicken body weight were determined on days 10, 20, 30, 40, 50, 60 and 75 p.i. Chicken mortality and trace element content in the liver were determined on day 75 p.i.

Chickens were killed by CO_2 inhalation and the alimentary tract was opened longitudinally from the gizzard to the cloaca. The contents were washed into a 100 μm sieve, transferred to a Petri dish, examined for the presence of a immature and mature *A. galli* under a microscope and the number of worms counted. Liver samples were dried at 100°C for 24 h, weighed, ground and then burned slowly in a muffle furnace up to 480°C for 48 h. The ashes obtained were treated with a mixture of concentrated H_2SO_4 and HNO_3 (1:5) in a sand bath and the wet residues were dissolved in 1 M HCl. The determination of Zn, Mn and Co was made using an atomic absorption spectrophotometer Varian Techtran model AA 220 (Anon., 1982). Data were tested using an analysis of variance (Steel & Torrie, 1980). Duncan's new multiple range test was used to separate significant differences between the means (Duncan, 1955).

Mathematical model

In order to study the development of *Ascaridia galli* populations, the infected organ (the intestine) of the host is viewed as a unique 'cultivator' and it is assumed that the therapeutic agent (the glycine–metal compounds) is supplied evenly and constantly into it. The compounds 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 passage of fluid through the alimentary tract of the chicken. It is assumed that the nematodes and the therapeutic agent are distributed evenly and form an homogeneous mixture as this allows differential equations to be used.

Following infection with *A. galli*, the number of larval and adult nematodes decreases as a result of an immune response by the host. This process may be described in 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 ($[\nu] = [day^{-1}]$) is the relative reduction rate constant.

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

The weight of healthy chickens (group 0) increases almost linearly as indicated by experimental data and this is in good agreement with data provided by the Dutch Company Eurybrigh regarding the development of hybrid Leghorn birds (Hisex white hybrid, Eurybrigh–Holland) during the first 20 weeks after hatching. A nutritional substrate is not regarded as a limiting factor. Thus, 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 ($[\mu] = [\text{g day}^{-1}]$) is the relative rate of gain in body weight.

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

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

The kinetics of worm establishment in the intestine of chickens and of the gain in body weight without or with treatment can be presented by the following ordinary non-linear differential equations:

$$\frac{dS_j}{dt} = \frac{\psi S_{j\,0}^2 - \beta_j S_j^2}{2S_{j0}} \quad j = 2,3,4,5,6,7 \tag{1}$$

$$\frac{dN_1}{dt} = -\nu N_1 \tag{2a}$$

$$\frac{dN_l}{dt} = -\nu N_l - a_l S_l N_l \quad l = 2,3,4,5 \tag{2b}$$

$$\frac{dN_m}{dt} = -\nu N_m + b_m S_m N_m \quad m = 6,7 \tag{2c}$$

$$\frac{dP}{dt} = \mu \tag{3a}$$

$$\frac{dP_i}{dt} = \mu + \mu_s - k_i N_i \quad i = 1,2,3,4,5,6,7 \tag{3b}$$

under the initial conditions:

$$t_0 = 0,\ S_j(t_0) = 0,\ N_i(t_0) = N_0,\ P_i(t_0) = P_0 \tag{4}$$

where t_0 is the time moment corresponding to the day of infection. We postulate: $t_0 = 0$. S_j ($j = 2, 3, 4, 5, 6, 7$) are the quantities of zinc, manganese and cobalt from the glycine–metal compounds, either in the form of chlorides or sulphates, in the worm biomass, respectively for groups 2, 3, 4, 5, 6 and 7, at a given time t, S_{j0} are the quantities entering the host's alimentary tract (the average daily dose of Zn^{2+}, Mn^{2+} and Co^{2+} respectively, taken up by each chicken from the food), ψ ($[\psi] = [\text{day}^{-1}]$) is the flow or dilution rate constant, determined as an average flow of passage through the alimentary tract divided by the average daily dose of compounds, $\beta_j = \psi + \alpha_j$, where α_j is the rate constant of the resorption of compounds in the host's intestine (the resorption constant). α_2 corresponds to $2Gly.ZnCl_2.2H_2O$, α_3 to $Gly.ZnSO_4.5H_2O$, α_4 to $2Gly.CoCl_2.2H_2O$, α_5 to $Gly.CoSO_4.5H_2O$, α_6 to $2Gly.MnCl_2.2H_2O$ and α_7 to $Gly.MnSO_4.5H_2O$; a_l ($[a] = [\text{day}^{-1}]$) are rate constants of decreasing worm populations due to the influence of zinc and cobalt compounds; b_m ($[b] = [\text{day}^{-1}]$) are rate constants of stimulating the nematode population by manganese compounds. The parameters k_i ($[k] = [g\ \text{day}^{-1}]$) are the relative rate constants due to the influence of *A. galli* infections and complexed compounds on the gain in host body weight. The parameter μ_s is the relative rate of gain in body weight resulting from cell stimulating processes.

Equation 2a describes the establishment of the worm population in experimental group 1; equation 2b describes the establishment of worm populations in experimental groups 2, 3, 4 and 5 respectively; equation 2c describes the establishment of worm populations in experimental groups 6 and 7. Equation 3a presents the gain in host body weight for control (group 0) and likewise, equation 3b for other groups.

For equations 1, 2 and 3 under conditions (4) the following analytical solutions are obtained:

$$S_j = S_{j\,0}\frac{\sqrt{\psi}(1 - e^{-\sqrt{\psi}\beta j\ t})}{\sqrt{\beta_j}(1 + e^{-\sqrt{\psi}\beta j\ t})} \quad j = 2,3,4,5,6,7 \tag{5}$$

$$N_1 = N_0 e^{-\nu t} \tag{6a}$$

$$N_l = N_0 2^{2\theta}\frac{e^{-(\nu+\theta\sqrt{\psi}\beta l)t}}{(1 + e^{-\sqrt{\psi}\beta l\ t})^{2\theta}} \quad l = 2,3,4,5 \tag{6b}$$

$$N_m = N_0 2^{-2\sigma} e^{-(\nu-\sigma\sqrt{\psi}\beta m)t}(1 + e^{-\sqrt{\psi}\beta m t})^{2\sigma} \quad m = 6,7 \tag{6c}$$

$$P = P_0 + \mu t \tag{7a}$$

$$P_1 = P_0 + (\mu + \mu_s)t - \frac{kN_0}{\nu}(1 - e^{-\nu t}) \tag{7b}$$

$$P_l = P_0 + (\mu + \mu_s)t - \frac{2^{2\theta} kN_0}{\nu + \theta}(1 - e^{-(\nu+\theta\sqrt{\psi}\beta l)t}) \quad l = 2,3,4,5 \tag{7c}$$

$$P_m = P_0 + (\mu + \mu_s)t - \frac{2^{-2\sigma} kN_0}{\nu - \sigma}(1 - e^{-(\nu-\sigma\sqrt{\psi}\beta m)t}) \quad m = 6,7 \tag{7d}$$

where $\theta_l = \frac{a_l S_{l0}}{\beta_l} \quad l = 2,3,4,5$

and $\sigma_m = \frac{b_m S_{m0}}{\beta_m} \quad m = 6,7$

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The solutions are: (7a) for group 0; (6a) and (7b) for group 1; (5), (6b) and (7c) for groups 2, 3, 4 and 5; (5), (6c) and (7d) for groups 6 and 7.

The immunological constant ν may be determined from the solution (6a) after taking in a logarithm:

$$\ln N_1 = \ln N_0 - \nu t \quad (8)$$

This is an equation with a straight line and an angular coefficient ν. Using the values of N_1, determined by the experiment, a plot of ln N_1 as a function of time can be constructed.

Results and Discussion

Data on the mortality of control and experimental chickens are presented in table 1. Survival was lowest in infected, untreated chickens from group 1 where a 50% mortality was observed. Statistically significant differences, using a Student's t-test, were established between group 0 and each of other groups (table 1). It should be noted that Gabrashanska *et al.* (2002a) found no statistically significant differences between the infected untreated group 1 and groups treated with cobalt–glycine compounds. However, such a difference ($P < 0.05$) was established between group 1 and the group treated with the mixed basic salt $(Zn_xCo_{1-x})_4.(OH)_6.SO_4.2H_2O$. The action of basic salts on the host is prolonged and with a lower toxicity compared with neutral salts (Gabrashanska *et al.*, 1993, 1999a; Gabrashanska & Timanova, 1995; Galvez-Morros *et al.*, 1995. Moreover, the stimulating action of Zn^{2+} from the mixed basic Zn–Co salt takes place. Thus, this could provide a further optimal therapy for chickens with helminthiases, including the simultaneous use of complexed (metal–amino acid or metal–protein) compounds and basic mixed salts.

With reference to the equation 1, dS/dt is the total change of the quantity S with time, where dS/dt increases with the salt flow $\psi S_0/2$ entering the alimentary tract of the host and decreases with the flow $[(\psi + \alpha)/2](S^2/S_0)$ which is exerted from the alimentary tract. The exiting salt flow is proportional to the square of the salt concentration in the alimentary tract at a given moment t, divided by S_0. Such a form of dependence, i.e. the square of the concentration is justified by a good agreement of the solutions (5) of equation 1 with experimental data relating to the establishment of *A. galli* in the alimentary tract and to avoid complex calculations connected with special functions, which should be appear by the solving of equation 3b.

The experimental and theoretical results of the change of *A. galli* populations in the host with time are shown in fig. 1. The mean ±SD for each group was calculated and data were compared using the Student's t-test, especially on day 75 p.i. (table 1). In all cases worm numbers decrease and this is likely to be due to the effect of immune and allergic reactions of the host leading to the elimination of some helminth parasites (Bykoryukov & Tachistov, 1965). It is seen (fig. 1) that in groups 6 and 7, treated with manganese compounds, higher levels of worm burdens were recorded

Table 1. The relationship between the number of *Ascaridia galli* and chicken body weight, mortality and trace metal contents in chicken liver at day 75 post-infection.

Chicken group	Chicken status and treatment	Number of worms	*P*	N	Mortality %	*P*	Chicken weight	*P*	Trace elements			
									Zn	Co	Mn	
0	Healthy	-	$P_{12} < 0.001$	28	6.67	$P_{01} < 0.001$	377.27 ± 29.1	$P_{01} < 0.001$	P_{01}	< 0.001	< 0.01	< 0.001
1	Infected	50.72 ± 7.18	$P_{13} < 0.01$	15	50	$P_{12} > 0.1$	277.95 ± 16.3	$P_{12} < 0.001$	P_{12}	0.01	> 0.1	< 0.01
2	2Gly.$ZnCl_2$.$2H_2O$	31.87 ± 10.02	$P_{14} < 0.001$	19	36.7	$P_{13} > 0.1$	314.89 ± 21.6	$P_{13} < 0.01$	P_{13}	< 0.01	> 0.1	< 0.01
3	Gly.$ZnSO_4$.$5H_2O$	41.4 ± 8.54	$P_{15} < 0.001$	20	33.3	$P_{14} > 0.1$	348.88 ± 10.5	$P_{14} < 0.001$	P_{14}	> 0.1	< 0.001	< 0.1
4	2Gly.$CoCl_2$.$2H_2O$	30.4 ± 11.95	$P_{16} < 0.001$	19	36.7	$P_{15} > 0.1$	305.60 ± 36.1	$P_{15} < 0.001$	P_{15}	> 0.1	< 0.05	< 0.01
5	Gly.$CoSO_4$.$5H_2O$	34.6 ± 3.27	$P_{17} < 0.01$	20	33.3	$P_{16} > 0.1$	319.15 ± 28.9	$P_{16} < 0.001$	P_{16}	> 0.1	> 0.1	< 0.001
6	2Gly.$MnCl_2$.$2H_2O$	65.46 ± 11.97	$P_{23} < 0.01$	19	36.7	$P_{17} > 0.1$	358.89 ± 14.3	$P_{17} < 0.01$	P_{17}	> 0.1	> 0.1	< 0.001
7	Gly.$MnSO_4$.$5H_2O$	58.46 ± 5.20	$P_{45} > 0.1$	18	40	$P_{23} > 0.1$	324.53 ± 11.87	$P_{23} < 0.01$	P_{23}	> 0.1	< 0.1	> 0.1
			$P_{67} < 0.05$			$P_{45} > 0.1$		$P_{45} > 0.1$	P_{45}	> 0.1	< 0.01	> 0.1
			$P_{35} < 0.01$			$P_{67} > 0.1$		$P_{67} < 0.05$	P_{67}	> 0.1	> 0.1	> 0.1

N, number of chicken examined.

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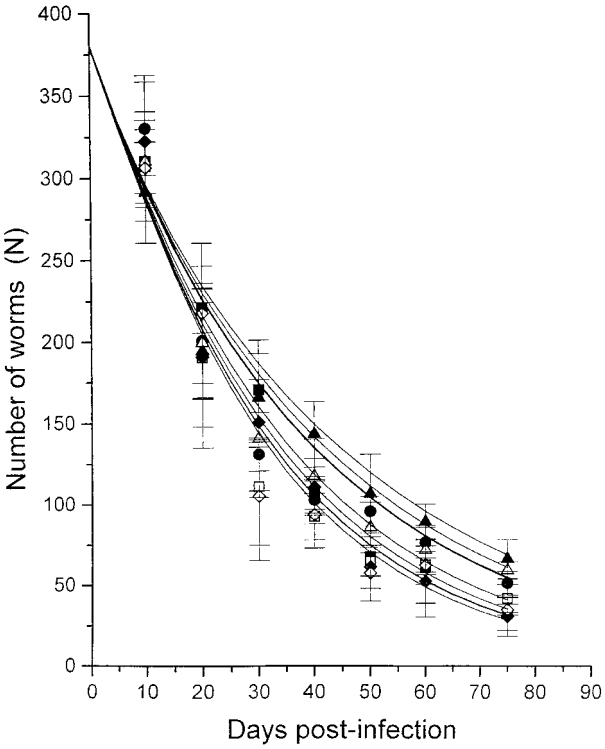

Fig. 1. The establishment of *Ascaridia galli* in the alimentary tract of seven groups of Hisex chickens (theoretical curves and experimental points). ●, group 1; ■, group 2; □, group 3; ◆, group 4; ◇, group 5; ▲, group 6; Δ, group 7. (See text for details of groups.)

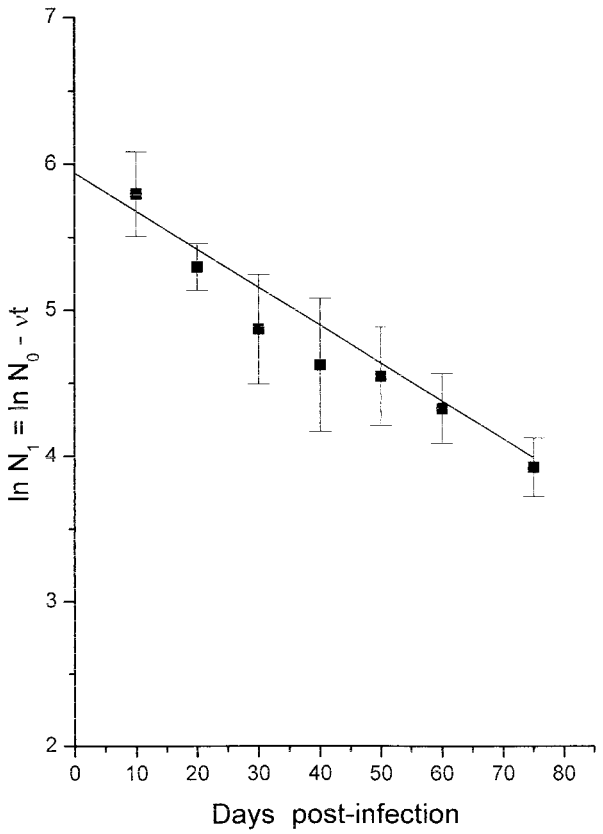

Fig. 2. Determination of the phenomenological constant of the host immune response ν on the basis of equation (8) using experimental measures of *Ascaridia galli* numbers in chicken group 1 – infected untreated chickens.

compared with untreated chickens from group 1. These differences are statistically significant (table 1). Manganese is known to increase bird growth and correct skeleton deformation caused by ascaridiasis (Berenschtein, 1968; Watson *et al.*, 1970) and manganese appears to stimulate nematode growth. In addition, the availability of manganese from Mn^{2+}/protein is higher compared with inorganic Mn salts (Black *et al.*, 1984; Smith *et al.*, 1995; Gabrashanska *et al.*, 1999b). An increase in *A. galli* worm burdens with manganese salt treatment was also noted by Teodorova & Gabrashanska (2002). Statistically significant differences in worm numbers were observed between groups 2 and 3 treated respectively with $2Gly.ZnCl_2.2H_2O$ and $Gly.ZnSO_4.5H_2O$ (table 1).

The theoretical solutions (6a), (6b) and (6c) are in a satisfactory agreement with experimental data and support the concept that the *A. galli* population in the host intestine decreases exponentially. The phenomenological constant of the immune response ν, which could be accepted as a characteristic phenomenological parameter in host–parasite systems, was calculated on the basis of experimental results. According to equation (8), ν is the angular coefficient of the straight line (fig. 2). The initial value $N_0 = 380$ was also calculated from fig. 2 ($\ln N_0 = 5.94$). For ν we obtained:

$$\nu = 0.026 \text{ day}^{-1} \quad (9)$$

This is the same value obtained by Gabrashanska *et al.* (2002a), where similar doses of 1450 embryonated *A. galli* eggs were used. The value $\nu = 0.0167$ day^{-1} was calculated for chickens infected with 450 embryonated *A. galli* eggs in a previous study (Gabrashanska *et al.*, 1999a). The difference between the values of the immunological constant ν is likely to be due to the different degrees of infection: a higher infection stimulates a stronger immune response. In addition, a heavy worm infection causes more unfavourable conditions for worm development and a higher rate of elimination.

Figure 3 demonstrates an increase of host body weight with time. The growth of infected untreated chickens (group 1) was considerably reduced in comparison with that of healthy control chickens ($P_{01} < 0.001$ on day 75 p.i, table 1). The addition of zinc, cobalt and manganese compounds in pharmacological doses to the diet increased the body weight gain in treated chickens and compensated to a certain degree the loss of body weight caused by *A. galli* infections. The highest weight gain among treated groups was shown in chickens from group 6, treated with $2Gly.MnCl_2.2H_2O$ (fig. 3, table 1). Manganese is an important antioxidant, activates numerous enzymes and plays a role in protein, carbohydrate and fat metabolism. Manganese is also nessesary for blood sugar regulation, sex hormone production, normal

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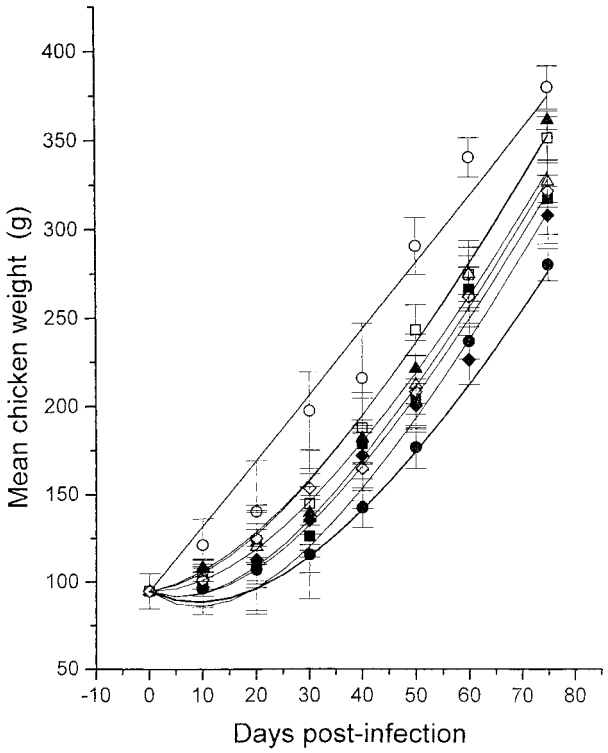

Fig. 3. Time course of the mean body weight in control chickens (○, group 0), chickens infected and untreated (●, group 1) and chickens infected with *Ascaridia galli* treated with 2Gly.$ZnCl_2$.$2H_2O$ (■, group 2), Gly.$ZnSO_4$.$5H_2O$ (□, group 3) 2Gly.$CoCl_2$.$2H_2O$ (◆, group 4), Gly.$CoSO_4$.$5H_2O$ (◇, group 5), 2Gly.$MnCl_2$.$2H_2O$ (▲, group 6) and Gly.$MnSO_4$.$5H_2O$ (Δ, group 7) up to 75 days p.i. (model solutions and experimental points).

skeleton and development and a healthy immune system (Deshmukh, 2001). A further point, concerning gain in body weight, relates to group 3 chickens treated with Gly.$ZnSO_4$.$5H_2O$. There is a significant difference ($P < 0.01$) between groups 2 and 3, between treatments with 2Gly.$ZnCl_2$.$2H_2O$ and Gly.$ZnSO_4$.$5H_2O$, respectively (table 1). This result supports that of Wedekind & Baker (1990), who supplemented zinc in poultry diets in the form of zinc sulphate, chloride and oxide, and observed that the availability of zinc in ZnO and $ZnCl_2$ relative to $ZnSO_4$ was lower, based on both growth and Zn accumulation in the tibia. The higher availability of zinc in sulphate could also explain the higher worm numbers in chickens from group 3 compared with group 2, suggesting that Zn positively influences the nematodes.

The model appropriately explains experimentally observed time courses of body weight gain in chickens (fig. 3). The value of the relative rate μ of the weight gain of control chickens was calculated by Gabrashanska *et al.* (2002a):

$$\mu = 3.7\,\mathrm{g\ day^{-1}} \qquad (10)$$

and this value is in very good agreement with the data in the present study.

Using parameter μ_s, a stimulating effect is taken into account. The living organism enters a characteristic state with higher metabolism and physiological activity after some unfavourable influences (such as high or low temperature, infections, intoxications, etc.). Popoff (1931) first determined stimulatory processes in plants expressed in enhanced growth resulting from an intensification of the metabolic network. Davtyan (1977) observed an increase of the redox processes and the activity of cholinesterases and hyaluronidases in the host with helminthiases. The present analyses relate to phenomenological aspects with little reference to 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. The theoretical results – solutions (7b), (7c) and (7d) are in a good agreement with experimental data (fig. 3).

Theoretical curves in figs 1 and 3 have been calculated using (9) and (10) under the following initial conditions: $S_{20} = S_{30} = 3.36\,\mathrm{mg}$, $S_{40} = S_{50} = 1.68\,\mathrm{mg}$, $S_{60} = S_{70} = 25.2\,\mathrm{mg}$, $N_0 = 380$ and $P_0 = 94.67\,\mathrm{g}$. The dilution rate constant $\psi = 0.08\ \mathrm{day^{-1}}$ was determined on the basis that the time required for the 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). We chose for the resorption constant $\alpha = 0.09\ \mathrm{day^{-1}}$ and a stimulation constant $\mu_s = 1.7\,\mathrm{g\ day^{-1}}$. These values were determined by the program 'Minuit' in our previous mathematical models (Gabrashanska *et al.*, 1999a, 2002a). The parameters a, b and k were calculated using the program 'Minuit' as follows: $a_2 = 0.0038$, $a_3 = 0.002$, $a_4 = 0.009$, $a_5 = 0.006$, $b_6 = 0.00021$, $b_7 = 0.0001$, $k_1 = 0.018$, $k_2 = 0.045$, $k_3 = 0.027$, $k_4 = 0.055$, $k_5 = 0.041$, $k_6 = 0.0115$, $k_7 = 0.014$.

Concentrations of zinc, cobalt and manganese in chicken liver are shown in figs 4, 5 and 6. Infected chickens showed significantly reduced levels of microelements compared with controls. Additional zinc, cobalt and manganese in pharmacological doses restored the liver deficiency of these elements. There were no statistically significant differences between trace elements levels in control and treated groups ($P_{02} > 0.1$, $P_{03} > 0.1$ for Zn; $P_{04} > 0.1$, $P_{05} > 0.1$ for Co; $P_{06} > 0.1$, $P_{07} < 0.1$ for Mn).

An improvement in the survival and body weight gain was observed in *A. galli*-infected chickens treated

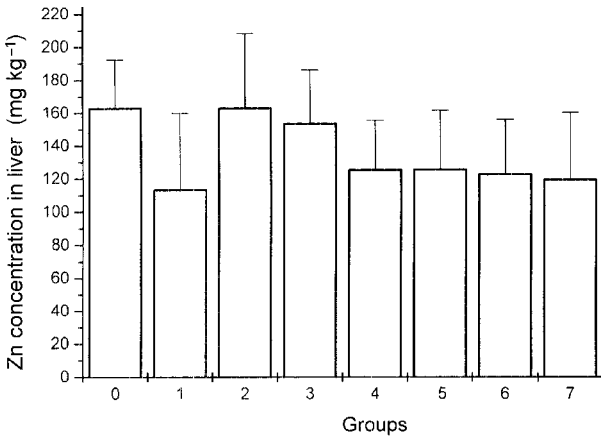

Fig. 4. Zinc (Zn) concentration in the liver of chickens from groups 0–7. (See text for details of groups.)

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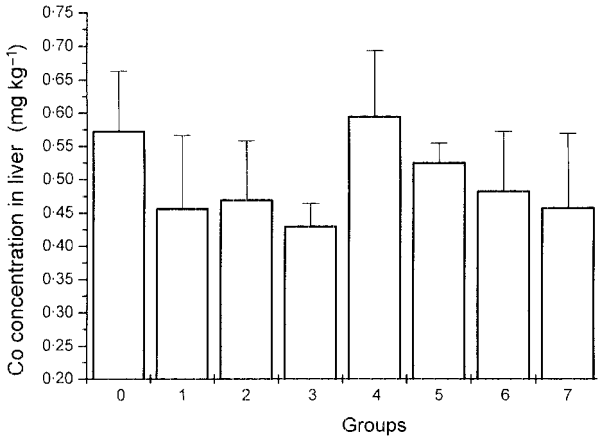

Fig. 5. Cobalt (Co) concentration in the liver of chickens from groups 0–7. (See text for details of groups.)

with complex compounds (fig. 3). In addition, $Gly.ZnSO_4.5H_2O$ and $2Gly.MnCl_2.2H_2O$ increased the gain in host weight to a high degree. Therefore these compounds showed a higher bioavailability compared with $2Gly.ZnCl_2.2H_2O$ and $Gly.MnSO_4.5H_2O$ and the differences are statistically significant (table 1). The concentrations of $2Gly.ZnCl_2.2H_2O$ and $Gly.MnSO_4.5H_2O$ respectively, in the liver were somewhat higher compared with Zn sulphate and Mn chloride but these differences are not statistically significant (fig. 4 and 6; table 1).

The present results show that zinc, manganese and cobalt supplementation in the form of complex compounds *per os* have a significant positive effect on infected chickens with respect to trace element deficiency. In previous studies it was shown that complex compounds of manganese and cobalt may improve bioavailability of minerals for both healthy and infected animals and that bioavailability is greater in infected chickens than in those with a normal microelement content (Gabrashanska *et al.*, 1999b, 2001, 2002a). Complexed trace elements compounds may be utilized

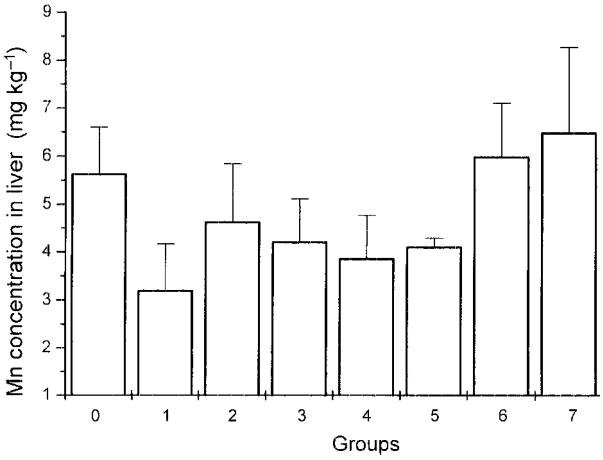

Fig. 6. Manganese (Mn) concentration in the liver of chickens from groups 0–7. (See text for details of groups).

better when there is a mineral depletion in the host. The present investigation is in a good agreement with other authors concerning cobalt (Southern & Baker, 1981; Kratzer & Vohra, 1986) and manganese (Smith *et al.*, 1995). The effective action shown by zinc, cobalt and manganese in these complex compounds is also similar to data for zinc and copper (Aoyagi & Baker, 1993). Flachowsky (1997) noted a greater effect of zinc complex compounds on infected pigs compared with healthy ones. The improved utilization of trace elements from complex compounds allows food to contain lower levels of these elements and this in turn reduces the risk of environmental contamination.

In conclusion, as an optimal therapy of chickens with helminthiases a recommended treatment would be to simultaneously include the use of zinc, manganese, and cobalt complex compounds in addition to the basic mixed Zn–Co salt (Gabrashanska *et al.*, 2002a) and copper basic salt $Cu_2(OH)_3Cl$ (Teodorova & Gabrashanska, 2002), because zinc stimulates immunity (Goyer, 1996) and copper has an antiparasitic action.

The mathematical model allows us to quantitatively describe *A. galli* populations under the regulation of host immune responses and to explain any changes in the gain in body weight of hosts untreated or treated with complex compounds. The fact that this mathematical model is in good agreement with experimental data, using the phenomenological constant of the immune response of the host, ν and the relative rate μ for body weight gain suggests that repeated time courses of *A. galli* establishment and host growth are well described by this model. Such a theoretical model could be used to investigate quantitatively the time course of infections and mean host body weights in a range of host–nematode systems (including low and heavy doses of infection) and also for estimating the efficiency of different therapeutic regimes.

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