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EFFECT OF DIFFERENT CONTENTS OF PROTEINS AND VITAMIN B_2 IN THE FEED ON THE PREVALENCE AND INFECTION INTENSITY OF *ASCARIDIA GALLI* IN CHICKENS

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ABSTRACT. A group of 10-day-old chickens was experimentally infected with a dose of 500 infective eggs of *Ascaridia galli* (Schrank, 1788) (Nematoda). Forty-nine days post infection the chickens were necropsied and the infection intensity and prevalence were determined. The group maintained on the feed containing 11% of protein showed higher infection intensity and prevalence compared to the chickens fed a diet containing 19% of protein. The addition of vitamin B_2 to the feed containing 11% protein resulted in lower intensity and prevalence of the infection. In both series of experiments no effect of Zn-bacitracin or vitamin B_2 on infection intensity or prevalence was observed for chickens fed feed containing 19% of protein.

Key words: *Ascaridia galli*, infection intensity, prevalence, vitamin B_2, Zn-bacitracin.

INTRODUCTION

It has been demonstrated that the composition of the host's diet is one of the factors influencing the development of the host-parasite system (Dubinsky and Rybos 1978; Bundy and Golden 1987; Mansour et al. 1991, 1992; Michael and Bundy 1991, 1992). In addition to the above papers, relatively little data is found in the subject literature on the influence of the diet on infection parameters of *Ascaridia galli* (Schrank, 1788) in chickens. The majority of these few papers focus on the effect of protein contents in the feed of chickens and hens upon those parameters (Ikeme 1971; Dubinsky and Rybos 1978; Żółtowska et al. 1991, 1995a, b, 1996; Permin et al. 1998).

The fact of bio-synthesis of vitamin B_2 by micro-flora of the alimentary tract of vertebrates is commonly known. It has also been known that the deficiency of vitamin B_2 in feed triggers characteristic symptoms of avitaminosis in chickens (Chou et al. 1971). In case of farm production, antibiotics are commonly used. According to Coates et al. (1968) they affect the vitamin management in chickens. In view of the above, verification of the role of Zn-bacitracin in the course of ascaridiosis in chickens may have both cognitive and practical importance.

The aim of this study was to follow the course of experimental ascaridiosis, under conditions of known effects of different protein levels in the feed and hitherto unknown effects of vitamin B_2- and Zn-bacitracin feed supplementation. The chickens were infected with eggs of *Ascaridia galli* (Nematoda). The present work focused on determining the prevalence and the infection intensity. The results obtained will permit better understanding of the host-parasite system formation under the assumed experimental conditions. This may have a cognitive value in parasitology and may provide important information for poultry production practice.

MATERIALS AND METHODS

The experiment was carried out in two series, applying identical conditions of rearing in mesh cages and the assumed experiment parameters. Both series (spring and winter) were split into 16 groups of 7 chickens in each.
The experimental material consisted of crossbreed Rhode Island Red and Rhode Island White chickens called Astra S. The groups of 10-day-old chickens, denoted by odd numbers were infected with a dose of 500 infective eggs of *A. galli* each using a rubber probe inserted into the crop. The infective eggs were obtained from the authors' own laboratory culture. The eggs dissected from *A. galli* females were maintained on Petri dishes in tap water at 22–25°C. Three times a week they were flushed with aerated water until the infective forms were obtained. The even-number groups of chickens were not infected.

Prior to the infection all animals were given balanced feed containing ca. 19% of protein. Throughout the experiment the chickens received feed and water *ad libitum*. Starting on the day they were infected, the chickens in groups numbered 9 through 16 were given unbalanced feed containing ca. 11% of protein, while groups 1 through 8 were given feed containing 19% of protein. Feeds for chickens groups 1–4 and 9–12, were supplemented with vitamin B_2, while groups 1, 2, 5, 6, 9, 10, 13, and 14 received Zn-bacitracin antibiotic. Thus, the experiment was planned according to the four-factor system 2 x 2 x 2 x 2, i.e. factor 1: infection of chickens with *A. galli*; factor 2: protein content in the diet; factor 3: addition of vitamin B_2; and factor 4: addition of Zn-bacitracin.

The components of the balanced and unbalanced feed were listed in Table 1. The table indicates that vitamin B_2 concentration in feed containing 19% of protein was 2.14 mg/kg, while in feed containing 11% protein it amounted to 1.248 mg/kg. Considering the daily feed (and vitamin B_2) consumption by chickens, groups 1–4 and 9–12 were additionally given this vitamin in quantities specified in Table 2, so that the overall quantity of that vitamin given to chickens reached the level equivalent to feed containing 4.5 mg/kg of it. As a result, the groups of animals fed the balanced feed and the unbalanced feed supplemented by vitamin B_2, were given the same dose of that vitamin. The

Table 1. Composition of experimental diets

Ingredient	Concentration: Protein [%] (g/100 g)	Concentration: Vitamin B$_2$ [mg/kg]	Diet 19% Content in feed: Feed [g]	Diet 19%: Protein [g]	Diet 19%: Vitamin B$_2$ [mg]	Diet 11% Content in Feed: Feed [g]	Diet 11%: Protein [g]	Diet 11%: Vitamin B$_2$ [g]
Wheat bran	14.2	3.4	160	22.72	0.544	0.0	0.00	0.00
Maize meal	9.2	1.4	330	30.36	0.462	130	11.96	0.182
Wheat meal	11.7	1.3	200	23.40	0.260	820	95.94	1.066
Soybean oil meal	44.2	3.3	245	108.29	0.808	0.0	0.00	0.00
Meat and bone meal	56.8	4.4	15	8.52	0.066	0.0	0.00	0.00
Fodder chalk	0.0	0.0	30	0.00	0.000	30	0.00	0.00
Phosphate	0.0	0.0	17	0.00	0.000	17	0.00	0.00
Fodder salt	0.0	0.0	03	0.00	0.000	3	0.00	0.00
Total in 1kg of feed	–	–	1000	193.29	2.14	1000	107.90	1.248
Total in 100g of feed	–	–	100	19.329	0.214	100	10.79	0.1248

weekly changes in the dose of vitamin B_2 administered were determined on the basis of the demands of chickens of different ages. In that way the established doses of vitamin B_2 and Zn-bacitracin were delivered in a form of aqueous solution, once a day into the crop. Feed mixes were prepared according to the nutrition standards effective in Poland. Zn-bacitracin antibiotic was administered in the lowest therapeutic dose (Table 2).

Table 2. The dose of vitamin B_2 (mg) and Zn-bacitracin (mg) administered as aqueous solution once a day in relation to age of chickens and kind of feed

Age (weeks)	Feed containing 19% of protein		Feed containing 11% of protein	
	Vitamin B_2	Zn-bacitracin	Vitamin B_2	Zn-bacitracin
2	0.104	2.0	0.123	2.0
3	0.130	2.5	0.152	2.5
4	0.156	3.0	0.183	3.0
5	0.208	4.0	0.244	4.0
6	0.260	5.0	0.305	5.0
7	0.312	6.0	0.366	6.0
8	0.338	6.5	0.396	6.5
9	0.364	7.0	0.427	7.0
10	0.390	7.5	0.457	7.5

The necropsies were performed on day 49 of infection. The intensity and the prevalence of the infection were determined according to the number of *A. galli* found in the intestines of the chickens.

The determined infection intensity data were subjected to statistical analysis according to Ruszczyc (1977). The significance of the difference between arithmetical means was calculated by analysis of variance using the "new range test". The standard deviation and variance ratio were also calculated.

RESULTS

Nematodes *A. galli* were not present in intestines of chickens representing groups which had not been experimentally infected. In view of the above, Table 3 presents the results related to the prevalence and infection intensity in odd groups subjected to infective eggs of *A. galli*. No other parasites were found in the alimentary tract of necropsied chickens.

In all groups, except groups 13sp and 13wi, no significant statistical difference between the arithmetic mean of infection intensity for the spring and the winter series were found, which was determined under identical experimental conditions (Table 3).

The results presented in Table 3 permit a conclusion that the protein content in the feed mix had the most extensive effect on both the intensity and prevalence of *A. galli* infection of chickens.

Table 3. Intensity and prevalence [%] of *Ascaridia galli* infection in chickens

Chicken groups [1]		1sp [2]	1wi [3]	3sp	3wi	5sp	5wi	7sp	7wi	9sp	9wi	11sp	11wi	13sp	13wi	15sp	15wi
Prevalence		14.29	14.29	28.57	28.57	28.57	42.86	14.29	28.57	85.57	100.00	100.00	100.00	85.71	100.00	100.00	85.71
Intensity (arithmetic mean)		0.286 ±0.756	0.143 ±0.378	0.286 ±0.488	0.286 ±0.488	0.286 ±0.756	0.429 ±0.535	0.286 ±0.756	0.429 ±0.787	3.143 ±2.734	4.000 ±2.769	4.000 ±2.082	4.000 ±1.736	19.000 ±11.839	12.000 ±8.124	10.000 ±6.351	11.000 ±8.206
Variant coefficient of intensity		264.6	264.6	170.8	170.8	264.6	124.7	264.6	183.6	87.0	69.2	52.0	34.6	62.3	67.7	63.5	74.6
Difference	1sp	–	0.14	0.00	0.00	0.00	0.14	0.00	0.14	2.86	3.71	3.71	3.71	18.71	11.71	9.71	10.71
Between	1wi	0	–	0.14	0.14	0.14	0.14	0.14	0.29	3.00	3.86	3.86	3.86	18.88	11.86	9.86	12.86
Group	3sp	0	0	–	0.00	0.00	0.14	0.00	0.14	2.86	3.71	3.71	3.71	18.71	11.71	9.71	10.71
Means	3wi	0	0	0	–	0.00	0.14	0.00	0.14	2.86	3.71	3.71	3.71	18.71	11.71	9.71	10.71
	5sp	0	0	0	0	–	0.14	0.00	0.14	2.86	3.71	3.71	3.71	18.71	11.71	9.71	10.71
	5wi	0	0	0	0	0	–	0.14	0.00	2.86	3.57	3.57	3.57	18.57	11.57	9.57	10.57
	7sp	0	0	0	0	0	0	–	0.14	2.86	3.71	3.71	3.71	18.71	11.71	9.71	10.71
	7wi	0	0	0	0	0	0	0	–	2.71	3.71	3.57	3.57	18.57	11.71	9.57	10.71
Level of	9sp	0	0	0	0	0	0	0	0	–	0.86	0.86	0.86	15.86	8.86	6.86	7.86
Significance	9wi	0	0	0	0	0	0	0	0	0	–	0.00	0.00	15.0	8.00	6.00	7.00
Difference	11sp	0	0	0	0	0	0	0	0	0	0	–	0.00	15.00	8.00	6.00	7.00
Between	11wi	0	0	0	0	0	0	0	0	0	0	0	–	15.00	7.00	6.00	6.00
Group	13sp	++	++	++	++	++	++	++	++	++	++	++	++	–	7.00	9.00	8.00
Means [4]	13wi	++	++	++	++	++	++	++	++	++	++	++	++	++	–	2.00	1.00
	15sp	++	++	++	++	++	++	++	++	+	+	+	+	++	0	–	1.00
	15wi	++	++	++	++	++	++	++	++	++	+	+	+	++	0	0	–

Explanations:

[1] Groups: 1, 3, 5, and 7 – factor 2A (protein content in the diet ca. 19%); 9, 11, 13, and 15 – factor 2B (protein content in the diet ca. 11%); 1, 3, 9, and 11 – factor 3A (addition of B_2 vitamin – Table 2); 1, 5, 9, and 13 – factor 4A (addition of Zn–bacitracin – Table 2); [2] sp – group in spring study; [3] wi – group in winter study; [4] 0 – insignificant difference between the arithmetic means, + – significant statistical differences ($p < 0.05$), ++ – highly significant statistical differences ($p < 0.01$).

The prevalence of *A. galli* infection in chickens of groups 1–7 fed the balanced feed did not exceed 28.57%. That means that out of 7 chickens exposed, not more than 3 were infected. In groups fed the unbalanced feed, in most cases the prevalence was 100%.

The infection intensity of chickens fed the balanced feed did not exceed 0.8. In case of chickens fed the unbalanced feed without the supplement of vitamin B_2 it was 3–4 (groups 9 and 11), and over 10 in case of groups fed that feed without the supplement of that vitamin (groups 13 and 15). The differences between arithmetic means for those 4 latter groups as compared to the other 48 were, in their vast majority, statistically highly significant and only in 7 cases – significant when compared to groups of chickens fed the balanced feed.

No evidence was found to confirm that administration of additional vitamin B_2 to chickens fed diet containing 19% of protein had any influence on the intensity or prevalence of the *A. galli* infection. The differences between arithmetic means of *A. galli* infection intensities for all groups of chickens fed the balanced feed were statistically insignificant. On the other hand, a distinct effect of vitamin B_2 in lowering the intensity of *A. galli* infection was found in chickens maintained on the low protein diet. During the studies conducted during both the first and the second series of experiments, a clearly lower numbers of *A. galli* were found in the intestines of chickens that were given additional vitamin B_2 (groups 9 and 11) compared to chickens receiving no additional dose of that vitamin (groups 13 and 15). In all cases the differences between arithmetic means of infection intensity between groups 9 and 11 as well as 13 and 15 were statistically significant or highly significant.

No straightforward effect of administration of Zn-bacitracin on the intensity or prevalence of *A. galii* infection in experimental chickens either in case of diversified protein diet nor in the case of presence or absence of supplementary vitamin B_2 was confirmed. Only in group 13sp receiving Zn-bacitracin and receiving no additional vitamin B_2, the arithmetic mean of infection intensity, amounting to 19, was statistically significantly higher as compared to all other groups in the experiment.

DISCUSSION

The fact that no *A. galii* specimens were found in chickens that were not experimentally infected, and that no other parasites were found in the alimentary tract permits a conclusion that the hygienic standards of keeping the chickens were appropriate.

The lack of statistically significant differences between arithmetic means of infection intensity in the groups of chickens treated with identical factors during the research procedures carried out in winter and in spring permits a conclusion that under the assumed conditions of the experiment the season of a year does not have a decisive effect on the process of *A. galii* infection.

The high variance ratio in infection intensities, amounting to as many as 264 in case of groups receiving the balanced diet and 34–87% in case of those fed low protein diet, contributed to the fact that no statistically significant differences were found between arithmetic means of infection intensity in groups of chickens fed vitamin B_2-supplemented feeds varying in the protein content (Table 1, groups 1 and 3 as compared to groups 9 and 11) despite apparent distinct differences. This gives evidence on a very high variability of results in the former case and a high variability – in the latter case. In that situation, to show statistically significant differences, the results of the means should be much higher, as it was observed in groups of chickens kept on low protein diet that were given no vitamin B_2 (Table 3, groups 13 and 14), as compared to the means for all other groups.

The lower intensity and prevalence of *A. galii* infection in chickens that were given the feed containing 19% of protein as compared to the animals fed feed containing 11% of that component (Table 3) is probably a consequence of the fact that birds properly fed are more immune and resistant to infection with that parasite. The higher susceptibility of chickens to infection was linked to their general weakening resulting from the unbalanced, low protein diet. Our observations confirm the results obtained by Ikeme (1971), Dubinsky and Rybos (1978) and Żółtowska et al. (1991; 1995 a, b; 1996). On the other hand the above-mentioned results are in contradiction to the results obtained by Permin et al. (1998). In case of experimental infection of laying hens with infective eggs of *A. galii* the latter authors observed that in case of birds fed feed of higher protein content the prevalence and intensity of the infection were higher as compared to the hens fed lower-protein-content feed. Permin et al. (1998) explained that phenomenon by the nutritive requirements of *A. galii*, which, when lower-protein-content feed is available, have limited feeding opportunities, which in turn leads to lower intensity and prevalence of the infection in hens.

The differences between results obtained by Ikeme (1971), Dubinsky and Rybos (1978), Żółtowska et al. (1991; 1995 a, b; 1996) and in the present study, as well as the results obtained by Permin et al. (1998) can be attributed to the fact that different age groups of hosts were studied (chickens or hens).The hens were studied by the latter author. Another reason behind the difference in the results may be the fact that in the former case the protein content in the diet of the chickens was 11% and 19%, respectively while in the diet of hens it was 14% and 18%, respectively. As a consequence, the results obtained in the experiment by Permin *et al.* (1998) are incompatible with the results obtained during the present study. This is not only the outcome of the differences in the protein content that in case of the balanced feed did not differ much, but particularly its quality, mainly in case of low protein feed (11% and 14%). Unfortunately Permin et al. (1998) do not specify the exact composition of the diets applied, giving only the concentrations of protein, fats, carbohydrates, and ash content. As a consequence, it is hard to compare the quality of the diet used in this experiment with the diet applied by Permin et al. (1998).

Feed containing 14% protein is much more complete than the feed containing 11% protein. During their growth, chickens need better-balanced feed to secure their growth and development of immunity processes. This was not offered by the feed containing 11% protein used in this study. On the other hand, hens studied by Permin et al. (1998), had their immunity systems already developed and they did not need a well-balanced feed for protection against parasites. On the other hand, *A. galii* specimens have better conditions for development under conditions of the alimentary tract of hens fed 19%-protein feed.

As early as in 1947 Acker et al. proved that the course of experimental infection with *A. galii* in chickens and hens depended on their breed, age, as well as on the dose and status of the infective eggs. On the other hand, Bundy and Golden (1987) believed that there were three major feeding factors influencing infection of the host by parasitic helminths: (1) changes in the immune system modified by the quality of feed, (2) nutritional deficiencies in the diet available to the parasites (3) changes to the host's intestinal environment caused by that diet. Those authors as well as the others, emphasize the special importance of the level and the quality of protein in the diet to the immune processes, which further affects the course of *A. galii* infection in hens and chickens. It should also be stressed that in case of these studies the feed fed to chickens containing 11% of protein was incomplete and in that case the nutritional deficiency available to the growing animals should result in general weakening, including compromising the still not fully developed immune system. In case of studies by Permin et al. (1998) the feed containing 14% of protein was complete and did not have any major influence upon the developed immune system of laying hens. On the other hand, lower availability of nutrients for *A. galii* could influence the intensity and prevalence of the infection through inhibition of parasite development.

Presently observed, distinct effect of vitamin B_2-supplementation had a clear influence on the decrease of intensity and prevalence of *A. galii* infection in chickens that were fed feed containing 11% of protein. The differences in arithmetic means of infection intensity between groups receiving vitamin B_2 and those fed feed without the supplement of that vitamin were statistically significant and highly significant (Table 3, groups 9 and 11 as compared to groups 13 and 15). As stated earlier, the quantity and quality of protein available in the feed, influence the development of immune processes in animals. Vitamin B_2 has an active role in the metabolism of proteins (Rivlin 1970, Chou et al. 1971). In view of the above, it is possible that vitamin B_2 positively affects the immune and resistance processes in the infected chickens fed the feed containing 11% of protein, and at the same time – the decrease in the intensity and prevalence of *A. galii* infection observed during these experiments. The feed containing 19% of protein is balanced in composition and in that case, with additional administration of vitamin B_2 no influence of that upon those processes was observed.

Absence of a clear effect of Zn-bacitracin administration on the intensity and prevalence of *A. galii* infection of chickens (Table 3) indicates that the antibiotic did not influence immunity and resistance in any significant way during the infection. An interesting fact is that in case of chickens of group 13sp fed the unbalanced feed supplemented with Zn-bacitracin, without vitamin B_2, the highest infection intensity, amounting to 19, was observed. The arithmetic mean of the infection intensity for chickens in this group was statistically very significantly higher than in all other groups in this study. The next highest infection intensity of 12 was recorded for group 13wi treated with the same factors as group 13sp. The latter result was close to the arithmetic means of infection intensity for groups 15sp and 15wi reaching 10 and 11 respectively. Groups 13sp and 13wi were given Zn-bacitracin while groups 15sp and 15wi were not given that antibiotic. In view of the above it may be assumed that Zn-bacitracin, in case of feeding chickens an unbalanced feed, may influence their higher susceptibility to the infection with *A. galii.*

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