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Influence on Oocyst Production of Concentration and Kind of Yeast in Rations of Rats Infected with Eimeria Nieschulzi

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INFLUENCE ON OOCYST PRODUCTION OF CONCENTRATION AND KIND OF YEAST IN RATIONS OF RATS INFECTED WITH EIMERIA NIESCHULZI [1, 2]

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Previous investigators have shown that a liberal supplement of yeast induces higher production of oocysts during an immunizing infection with *Eimeria nieschulzi* than either certain other supplements or complete lack of supplement. The question arises, however, whether lesser amounts of yeast would result in lower oocyst yields, and conversely, greater amounts in higher yields. Furthermore, because of the seeming association of the substance that produced the conditions requisite for higher oocyst counts with vitamin G, and sometimes B also, it was evident that a study with two strains of yeast differing in comparative content of vitamins B and G would be exceedingly relevant to a fuller understanding of the nature of this substance. These problems prompted this investigation.

HISTORICAL

This field of endeavor has only recently been opened and literature on the subject is not abundant. Becker and Morehouse (3) were the first to obtain a modification of the intensity of a coccidial infection by dietary means alone. After experimenting unsuccessfully with numerous types of rations to alter the quantitative aspects of the parasite's development, they finally discovered (4) that a ration that was designed to be deficient in vitamins B and G would entail a great decrease in numbers of oocysts discharged by infected rats. Continued work (5) seemed to indicate that the observed effect was due for the most part to vitamin G deficiency.

Later (6), (7) the same investigators made comparisons of the effect of different materials used as sources of vitamin G. There proved to be considerable variability in the results, for some substances rich in this vitamin, e.g. liver and skim-milk powder, were low in the hypothetical coccidium-promoting factor (called by them coccidibios). Yeast, on the other hand, furnished an equally good

1 Original thesis (M. S.) on file in the Iowa State College Library.
2 This study was carried on under Industrial Science Research Project 40.

supply of vitamin G and also favored the growth of the parasite to a maximum.

Becker and Derbyshire (2), continuing this line of endeavor, ran biological assays of various feeding stuffs for coccidibios. Their results definitely show that certain of these materials added to the basal ration are much more favorable to the development of *Eimeria nieschulzi* in the rat than others, regardless of whether a single coccidium-promoting substance is accountable.

METHODS

The technique outlined by Becker and Derbyshire was followed closely in this work. The microorganism used was *Eimeria nieschulzi* Dieben (1924) of the strain originally prepared by Becker (1). Methods of producing infections, handling the hosts, and collecting and enumerating the parasites have been recorded by the above authors. The "K-value" appearing in the tables is the ratio of the mean number of oocysts discharged by rats on a test ration to those passed by the controls. Similarly, the "W-value" is the ratio of the mean weight gain for the first 16 days of the test series to the same for the controls.

A control or reference ration was made up in parts as follows: Beet sugar, 69; unextracted casein, 15; celluflour (fine cellulose, inert ingredient), 3; complete salt mixture, 4; lard, 3; cod liver oil, 2; powdered yeast, 4. This ration provides good growth for the hosts and is quite favorable to the parasite. Test rations were made by adding or subtracting yeast, removing or adding sugar as the case demanded.

The yeast employed throughout was Fleischmann's dry, powdered variety. Two kinds were used: one containing 20 International B_1 units per gram and 12 G complex units; the other containing approximately 9 B_1 units and 20 G complex units per gram. The latter was used in all the rations except where a low G yeast was desired.

EXPERIMENTAL

The first series of experiments was designed to test the effect of different amounts of yeast in the ration ranging from none at all to 24%.

In table 1 is presented in condensed form, a summary of the findings of this phase of the study. In parentheses are the number of rats in the test series in each case; the reference series always contained equal, or slightly fewer, numbers.

Table I — W- and K-values for Rations Containing Different Proportions of Yeast

	Amount of Yeast in Ration					
	None (12)	1% (19)	2% (12)	4% (con)	8% (19)	24% (20)
W-value	0.273	0.490	0.841	1.00	0.774	0.696
K-value	0.175	0.392	0.788	1.00	1.085	1.184

To determine the statistical meaning of the results, the data of each experiment were subjected to an analysis of variance according to the method of Snedecor (8). F-values were obtained which were far beyond the point of 1% probability for the rations employing no yeast and 1% yeast. This indicates highly significant difference between the effects produced by each of these rations and the control. In the other cases non-significant F-values were obtained.

A sample of yeast was available having the same composition as that used in the preceding work except that it had been irradiated by the Fleischmann Company to increase its vitamin D potency.

A test ration was constructed using this yeast at the 4 per cent level. Eighteen rats on this ration discharged oocysts in millions ranging from a low of 49 to a high of 220, mean 136.5. The sixteen controls ranged from 90 to 234 millions, mean 134.3. These values resulted in a K-value of 0.984. The mean weight gains were likewise very close, a W-value of 1.150 being recorded. It was evident that there was no noticeable effect here.

The following investigations were undertaken in an effort to determine whether the vitamin G complex occurring in yeast imparted the coccidigenic properties to that substance. Two kinds of yeast were used as stated above.

Three experiments were performed comparing these yeasts at the 4 per cent, 3 per cent, and 2 per cent levels. Table 2 presents a summary of the data from these experiments.

In the experiment involving 3% yeast, the weight gains were so nearly alike that the animals were left on the diets an extra week to better determine the effect of the rations upon growth. At this time those on the low G ration had made a mean gain of 91.5 g.

*Table II — W- and K-values for Rations Containing Low B-high G Yeast, (1), and High B-low G Yeast, (2), Fed at 3 Levels**

	Level of Yest		
	4% (17)	3% (14)	2% (22)
W-value	0.943	0.962	1.008
K-value	0.857	0.980	1.170

* The K-and W-values are designated as the ratio of oocyst counts and weight gains respectively for the low G series to the same for the High G series, or (2) ÷ (1). The number of rats in the low G series in each experiment are shown in parentheses.

while those receiving high G yeast had made a mean gain of 81.6 g.

Some of the animals on each 2 per cent ration were likewise treated. Those receiving low G and high B made a mean gain of 83.3 g. while the hosts getting more G but less B only gained an average of 60.4 g. The W-value of 1.008 indicates that most of this difference came about after the first 19 days of experimental feeding. (In this experiment the preinfection period was lengthened to 12 days; thus the W-value is based on a 19-day growth period). An analysis of variance of these growth records reveals that this difference is highly significant.

DISCUSSION

From table 1 it is seen that there was a definite trend downward in oocyst counts from the rats receiving the most yeast to those receiving the least. The differences, however, were not in strict proportion, and the high-yeast rations apparently offered only slightly more favorable growth conditions to the parasite.

In the low-yeast rations, there was in every case a progressive decline in oocysts as the amount of yeast was decreased. These results offer conclusive evidence for the occurrence in yeast of a factor which is favorable to the development of coccidia. Furthermore, yeast is comparatively rich in this substance, since only 4 parts in the ration seem to confer almost the optimal environmental conditions for the parasite.

Where large amounts of yeast were used, and also at the 2 per cent level, the results were not significantly different from the controls. It seems logical to the writer, however, to suppose that these differences, though small, are real and are functions of the amount of yeast in the ration. In other words the downward trend of the K-values as the percentage of yeast drops, is not merely an accident of sampling but is the result of the differential action of the yeast.

An examination of the growth records in table 1 yields several facts of interest. In the first place it should be noted that the control ration containing 4 parts of yeast was a fortunate choice since it conferred the best growth conditions for the host. There is also evidence in support of the view of Becker and Morehouse (3) that parasite development is not merely a function of host growth, for rats on the 8 per cent and 24 per cent yeast rations made only 77 per cent and 70 per cent as much growth respectively as the controls, and yet the protozoan flourished more abundantly in both test series.

The experiments comparing yeasts low and high in vitamin G were attempts to clarify the relation, if any, between it and the hypothetical coccidium-stimulating substance. Table 2 shows that in no case was there wide variation in oocyst production between rats receiving low and high vitamin G in the yeast of the ration.

The earlier experiments bring out the fact that it was at the 2 per cent level, and lower, that discriminating effects of lowered concentrations of yeast became most apparent. It would seem, then, that with rations differing in the relative amounts of vitamins B and G in yeast, if either ration contained a preponderance of the coccidigenic factor, or factors, it would be noted when these yeasts were fed at the 2 per cent level. That no such action was evident leads to the conclusion that vitamin G itself is certainly not the factor responsible for the heightened coccidian development obtained when yeast is fed. This work confirms several proposals in the literature (2), (7) that the coccidistatic properties of a yeast diet low or lacking in vitamin G is not entirely due to the vitamin deficiency.

That there actually was a considerable difference in vitamin content of the two rations is brought out by the response of the rats to them. Those on a low G-high B ration made much greater weight gains over a 32-day observation period than the others getting more G and less B. This highly significant difference can not be considered due to the infections because both series of animals experienced infections of nearly equal intensity, as the K-value close to 1 shows. Indications are that as vitamin B was decreased, a growth factor was likewise depleted, the effects of the shortage becoming manifest about the 19th day.

Admittedly, the problem of the rôle played by these two vitamins in experimental infections of *Eimeria nieschulzi* is not entirely solved by attributing no effects to either of them, for it is well known that a shortage of the two entails lower parasite development. From the results of the present study, however, it seems valid to conclude that the observed phenomena are compatible with the hypothesis that there was in both yeasts approximately the same amounts of a substance, such as coccidibios, which affected the growth of the parasite but not that of the hosts.

Conclusions

There is a direct relation between the amount of yeast supplement added to a basal ration and the number of oocysts discharged by infected rats. The latter value increases as yeast concentration goes up.

Irradiation of yeast as a source of additional vitamin D in an adequate ration is without effect in altering the quantitative aspects of a coccidian infection.

Evidence gathered from work involving the use of yeasts low and high in vitamin G points to the separate entity of that vitamin and the hypothetical coccidium-growth-promoting substance.

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