EFFECTS OF LOW-LEVEL INFECTIONS BY COCCIDIA AND ROUNDWORMS ON THE NUTRITIONAL STATUS OF RATS FED AN ADEQUATE DIET\textsuperscript{1,2}

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Summary

Experiments were conducted on male, Sprague-Dawley rats of the same age fed a standard laboratory diet to determine the comparative effects of single and concurrent subclinical infections by coccidia (Eimeria nieschulzi) and roundworms (Nippostrongylus brasiliensis) on nutritional status and metabolism. All infections produced anorexia, but it was more prolonged and intense with the concurrent ones. Feed:gain ratios were increased only by infections by nematodes alone, but average daily gains were reduced beyond the effect of anorexia (i.e., "specifically") only with the double infections. Infections by coccidia only, but not by nematodes only, specifically reduced the apparent digestibility of dry matter, organic matter and N; whereas only the apparent N digestibility was specifically reduced by the double infections. Balances of N were reduced in infected rats and pair-fed controls during anoretic periods, but significant differences between the daily means for infected and pair-fed rats occurred only erratically. Specific increases in urinary N loss did not occur with the single infections, but occurred on 2 d with the double ones. Increased losses of fecal N occurred with both coccidial and double infections, but they were much more pronounced in the latter. No effects of infection on the balances or carcass contents of Ca and P occurred. The only significant differences between mean empty carcass densities occurred with the double-infected and free-fed uninfected groups, with the mean for the uninfected group being the higher.

(Key Words: Nematodes, Coccidia, Rats, Diets, Nutrient Balances, Apparent Digestibilities.)

Introduction

Low-level, subclinical infections by nematodes and coccidia are almost ubiquitous in livestock on pasture in many regions of the world, and frequently occur concurrently in the same animal. The effects of these infections on productivity and carcass quality become more important as the profit margin in livestock production narrows. It is established that such infections by nematodes alone may produce serious, but not obvious, effects on carcass quality (Sykes and Coop, 1976), and "stress" may cause subclinical infections by coccidia to "break" and become clinical coccidiosis. In the absence of such stress, however, subclinical infections by coccidia are often regarded as essentially innocuous.

The present experiments were designed to test, in a rat-parasite system with a nutritionally-adapted diet, the hypothesis that subclinical infections by coccidia are of much more concern when superimposed on a nematode infection than when they occur alone. Confirmation of this hypothesis in this model would suggest that high prevalence in a herd of livestock of such double infections indicates the
advisability of chemotherapy in the absence of frank signs of disease, provided that the results are applicable by extension to larger non-ruminants and to ruminants.

Materials and Methods

Three experiments were conducted, one each with rats infected with coccidia (Eimeria nieschulzi) and nematodes (Nippostrongylus brasiliensis) alone, and one with rats infected with both parasites concurrently. Both parasites inhabit the anterior to middle portions of the small intestine. All rats were Sprague-Dawley males 70 d of age on the date the experiment began, an age when Ca deposition in the skeleton occurs at a rapid rate (Sherman and MacLeod, 1925).

The rats were allocated to four groups (A, B, C, D) in such manner that the mean weights of the groups were within 1 SD of each other and the differences between them were insignificant. In the experiment in which only coccidia were used for the infections, each group contained six rats. In the other experiments, each group contained eight rats. Group A rats were killed at the beginning of the experiment after 12 h of fasting, and their bled and eviscerated carcasses used to establish base values for Ca and P composition. All other rats were placed in metabolism cages for 1 wk to accustom them to the housing conditions before the beginning of the balance trials. All rats were fed from the same lot of commercial rodent diet each day.

Group B rats served as the free-fed, uninfected controls. On the day when Group C rats were inoculated with parasites, Group B rats were inoculated with the vehicle only via the same route.

Group C rats, each received one or both of (1) an aqueous suspension of six million sporulated oocysts of coccidia via gastric intubation and (2) a suspension in normal saline solution of 2,000 infective, third-stage nematode larvae injected sc. When both kinds of parasites were administered to the same rat, the oocysts were given on d 4 after the nematodes so that the latter would be present in the intestine at the time when the coccidia arrived there, thus simulating a situation wherein livestock already harboring a nematode infection acquired one by coccidia. The numbers of parasites administered to each animal would be expected to produce subclinical infections under normal conditions of nutrition and stress, based on experience in this laboratory using these strains of parasites in this strain of rats.

Group D rats served as uninfected, pair-fed controls for the correspondingly-numbered Group C rats, being inoculated with the vehicle only (water for the coccidia, normal saline for the nematodes) on the days when the pair-member received parasites. The schedule of events for Group D rats was set back 1 d so that each rat was presented with exactly the same amount of food each day that its Group C pair-mate had consumed the previous day.

Beginning with the day of inoculation of the Group C rats with parasites, all rats were on balance trial for the remainder of the experiment. On the day before inoculation, food was removed from each cage and each rat was weighed after 12 h of fasting. Another 12-h fast was imposed the night before killing each rat in order to obtain a final, fasted weight.

At the same hour each day, each rat was weighed, feed consumption was determined, and all ors and excreta were collected and measured. Urine was collected in tubes containing sufficient sulfuric acid to maintain the pH at approximately 3. It was then stored at 4 C until time of analysis. Feces were collected into folded-foil envelopes, weighed and then transferred to air-tight plastic envelopes for storage at -20 C until time of analysis.

All animals were killed after the period of peak oocyst production in the rats infected with coccidia had passed, or after a comparable period of time. Both of the species of parasites used produce infections that cure spontaneously, with peak egg and oocyst production before 12 d after infection (Ogilvie and Jones, 1971; Liburd, 1973). All carcasses were weighed, and the densities of the bled and eviscerated carcasses were determined via water displacement. They were then stored at -20 C in air-tight plastic bags until dissolved in nitric acid for subsequent determination of Ca and P (Field and Suttle, 1966).

Proximate analyses of each lot of feed were performed by the Alabama State Chemist's Laboratory using AOAC methods (AOAC, 1980). Apparent digestibilities of dry matter, organic matter and N were determined by

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5 Purina Rodent Chow, Ralston Purina Co., St. Louis, MO.
standard methods (Lindahl, 1959). Calcium and P were determined by the Auburn University Soil Laboratory via atomic absorption spectroscopy. Nitrogen was determined by ammonia electrode after digestion as for the standard Kjeldahl method (Bradstreet, 1965; Nube et al., 1980). The accuracy of this method of N determination was verified by frequently having duplicate samples analyzed by an independent laboratory using the complete, standard Kjeldahl method.

Analysis of variance, Student’s t-test (Snedecor and Cochran, 1967) and Duncan’s multiple range test (Duncan, 1955) were used to identify significant differences between sample means.

Results and Discussion

Feed Consumption. Differences in daily mean feed consumption between the groups of free-fed uninfected control rats in the experiment were insignificant. The mean daily feed consumptions per kg body weight of infected and double-infected rats are shown in figure 1. An anoretic day was defined as a day when the mean feed consumption of the infected group was significantly less than that of the uninfected, free-fed control group. Rats infected only with coccidia were anoretic on d 8 to 11 after inoculation with parasites, whereas those infected only with nematodes were anoretic only on d 8. Superimposing the infections produced a longer period of anorexia, 5 d, than

Figure 1. Mean daily feed consumption, as grams of dry matter (DM) consumed/kg body wt/day, for infected rats (Group C) in each experiment. Days correspond to those after inoculation of nematode larvae (AIL) in the infections including nematodes.
with the single infections, and increased its severity 1 d beyond that observed with rats infected with coccidia only. On that day the mean consumption of the double-infected rats was less (P<.001) than the lowest mean for the single-infected animals.

Roudabush (1937) demonstrated that the individual sporozoites of E. nieschulzi, which leave the oocyst to establish the infection, may penetrate the epithelial cells of the midportion of the small intestine any time within 4 d of the introduction of oocysts into the stomach. Consequently, all stages of the life cycle of the parasite from first generation merozoites through oocysts would have been present in the rats infected with coccidia in these experiments during the anoretic period, the first anoretic day corresponding to the time of development of oocysts derived from the first sporozoites that penetrated epithelial cells. In the double infections, anorexia continued through the day of peak oocysts production, d 9 after inoculation of oocysts (AIO), but in the single infections mean feed consumption on this day was essentially the same as that of the free-fed controls.

In rats infected with coccidia and with coccidia plus nematodes the anoretic period was followed by a period of appetite rebound. With both of these groups, the rebound period lasted 3 d. But with the single-infected rats there was only 1 d during this period when their mean, expressed as g/kg body weight, was significantly higher than that of the controls, whereas the mean of the double-infected rats were significantly higher for the remainder of the experiment.

Feed to Gain Ratios. With the coccidial and double infections there were no significant differences between group means nor were there significant differences between the group means of the free-fed uninfected controls. With the nematode infections there was a highly significant difference between the means of Group C (33.61) and Group D (2.065). Obviously, there was some interactive effect between the two parasites which improved the feed conversion efficiency in the infected rats to the point where it was not significantly different from that of the pair-fed controls. The nature of this interactive effect is unknown.

Gains in Body Weight. In the experiment with coccidia only, Group C had a negative mean average daily gain (ADG; −1.18 g), whereas the mean ADG for Group B was positive (1.56 g) and higher (P<.005). The difference between the means of Groups C and D was not significant.

In the experiment with nematodes only, the differences between group means were insignificant, but in the one with the double infections the mean for Group C (−.05 g) was less than that of Group B (4.72 g, P<.001) and Group D (1.15 g, P<.05). In all experiments the mean gains for Group B rats were essentially the same. Consequently, the effects of the double infections on ADG were specific—beyond those resulting from anorexia—whereas the single infections either did not affect the ADG (with nematodes) or reduced it simply through causing anorexia.

There were no significant differences between the mean final fasted weights of the groups in the experiments with the single infections. In the experiment with double-infected rats, the mean of Group C (284.26 g) was less than that of Group B (336.51 g, P<.005), but not significantly less than that of Group D (298.53 g). Consequently, the reduced ADG produced through anorexia in rats infected with coccidia only was not reflected in the mean final fasted weight because the increased consumption after the anoretic period led to increased ADG during the last days of the experiment. However, the mean final metabolic size (FMS; body wt in kg) expressed as a percentage of the initial metabolic size was less for Group C in this experiment (96.44%) than for Group B (104.97%, P<.005).

Infection by nematodes alone did not affect the mean FMS as a percentage of the initial metabolic size. In the experiment with the double infections, however, the mean FMS so expressed for Group C (99.83%) was less than that of both Group B (114.21%, P<.001) and Group D (103.66%, P<.025).

Davis et al. (1959a, b, 1960a, b) reported that the comparative effects on weight gains in calves of single and double infections by mixed species of coccidia and the nematodes Cooperia punctata, Ostertagia ostertagi, Trichostrongylus colubriformis and Strongylus papillosus varied according to the species of nematode. In contrast to the present study, these authors investigated only clinical infections and limited their observations to weight gains and clinical signs.

Apparent Digestibilities. The mean daily digestibility quotients for dry matter (DM), organic matter (OM), and N of Group B rats

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exceeded 80 and were essentially the same for each of the experiments. Decreases in the apparent digestibilities of DM (figure 2), OM (figure 3), and N (figure 4) were associated with infection by coccidia alone. During the period of appetite rebound after anorexia, the mean apparent DM digestibility of Group C rats was 91.8% (P<.001) and 94.8% (P<.05) of the mean for Group D on d 10 and 11, respectively. The mean apparent OM digestibility for Group C was less than that of Group D on d 9 to 12, the respective percentages of the latter mean being 94.2 (P<.01), 91.9 (P<.001), 95.1 (P<.05) and 97.5 (P<.01). On d 9 and 10 the mean apparent N digestibility of Group C rats was also below that of Group D rats, 86.5% (P<.01) and 73.4% (P<.001), respectively. Of the apparent mean digestibilities determined, only that of DM was lower in Group C than in Group B, but on the days concerned it was not significantly different from the mean of Group D. It is of interest that reduction of consump-

![Figure 2. Mean daily apparent dry matter digestibility quotients for infected rats (Group C) in each experiment. Days correspond to those after inoculation of nematode larvae (AIL) in the infections including nematodes.](image-url)
tion was not associated with an increase in apparent digestibility.

In the experiment with nematode infections only, the mean apparent DM digestibility of Group C was never significantly below that of Group D, although on d 6 and 8 it was below that for Group B (P<.05 and P<.025, respectively). Day 8 was anoretic for Group C rats, but d 6 was not. Only on d 10 was there a difference in the mean apparent OM digestibilities of Groups C and D (P<.001), with the mean for Group C 96.2% of that for Group D. The mean feed consumptions for both groups on this day were essentially the same. The mean apparent N digestibility fluctuated considerably from day to day, and no clear influence of infection on its value could be identified; consequently, these data were not included in figure 4.

In the experiment with the double infections the mean apparent DM digestibility for Groups B and C was essentially the same during the

![Graph showing mean daily apparent organic matter digestibility quotients for infected rats (Group C) in each experiment. Days correspond to those after inoculation of nematodes larvae (AIL) in the infections including nematodes.](image)
period when the latter group was anoretic. During the period of appetite rebound after anorexia, the Group C mean was higher than the Group B mean (104% P<.001). There was never a significant difference between the means of Groups C and D. With regard to mean apparent OM digestibilities, the only significant differences between group means occurred during the period of appetite rebound for Group C rats, when their mean was below that of Group B (96.2%, P<.005). In contrast to the situation described with the single infections, double infections were associated with a reduction in apparent N digestibility during the anoretic period, the mean for d 8 to 12 after inoculation of larvae (AIL) for Group C being 88.1% that of Group D (P<.005).

Infections by coccidia, therefore depressed the apparent DM and N digestibility during the period of appetite rebound after anorexia,
whereas no specific effect of infection on these characteristics occurred with nematode infections alone. Surprisingly, there was no specific effect of the double infections on DM digestibility, although the apparent digestibilities of both OM and N were reduced on certain days. With the double infections, the period of reduced apparent N digestibility occurred during anorexia, whereas it occurred during the period of appetite rebound after anorexia in the rats infected only with coccidia. As with the apparent DM digestibility, the double infections failed to reduce the apparent OM digestibility. Both of the single infections reduced the apparent OM digestibility relative to that in the pair-fed controls; the nematode infection on a single day after anorexia, the coccidial infection during the period of reduced feed consumption concurrent with anorexia. Double infections, each of corresponding level, therefore, appeared to have less effect on apparent DM and N digestibilities during increased feed consumption than did coccidial infections alone, but an effect greater than either infection alone on apparent N digestibility during a period of reduced feed consumption. The lack of a specific effect of the double infections on the apparent OM digestibility when both single infections reduced it is difficult to explain.

Sykes and Coop (1977) reported that the mean apparent N digestibility of sheep infected with the stomach worm Ostertagia circumcincta was significantly below that of pair-fed controls, indicating that abomasal parasitism has the same impact on N digestibility as does parasitism of the small intestine by coccidia and nematodes in rats. These same authors (Sykes and Coop, 1967), in contrast, reported no significant differences between the mean apparent N digestibilities of sheep infected with the small intestinal nematode Trichostrongylus colubriformis and pair-fed controls. In the present investigation, there were no significant differences in mean daily balances of Groups C and D rats, differences that would indicate a specific effect of infection, did not occur in the experiment with coccidial infections only, occurred during d 5 (Group C lower, P<.01), 7 (Group C higher, P<.025) and 8 (Group C lower, P<.025) in the experiment with nematodes only, and occurred on only one day, 15, in the experiment with the double infections, when the mean for Group C was higher (P<.025). Parkins et al. (1973) reported a significantly greater loss of urinary N in sheep infected with the stomach worm O. circumcincta than in pair-fed controls. In the present investigation, there were no significant differences in mean daily urinary N output, expressed as mg/kg body weight\(^{-75}\), between Groups C and D in the experiments with the single infections, but in the experiment with double infections the means for Group C were higher than for Group D on d 12 (P<.05) and 14 (P<.025; table 1). Roseby and Leng (1974) reported that an increase in the rate of excretion of urea in the urine was responsible for a significant increase in the urinary N of sheep infected with T. colubriformis. Horak and Clark (1964) reported an increased fecal loss of N in calves infected with O. ostertagi, but their design did not include pair-fed controls to correct for the effects of anorexia. In the present study, the mean daily N absorption, expressed as percentage of daily intake per kg body weight\(^{-75}\), was consistently reduced by infection only in the double-infected rats, where on d 5, 6, 7 and 8 all it was less than in the pair-fed controls (table 1). In the rats infected with coccidia only, there were no significant differences in mean N absorption between infected and pair-fed groups. In the experiment with nematodes
TABLE 1. MEAN DAILY NITROGEN BALANCES (G/KG BW \textsuperscript{-0.75}) IN DOUBLE-INFECTED RATS (GROUP C) AND PAIR-FED CONTROLS (GROUP D)

<table>
<thead>
<tr>
<th>Day (ALL)</th>
<th>Intake N</th>
<th>Fecal N</th>
<th>Urinary N</th>
<th>Absorbed N (%)</th>
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<tbody>
<tr>
<td></td>
<td>Grp C</td>
<td>Grp D</td>
<td>Grp C</td>
<td>Grp D</td>
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<tr>
<td>4</td>
<td>2.365</td>
<td>2.132</td>
<td>.417</td>
<td>.460</td>
</tr>
<tr>
<td>5</td>
<td>1.673</td>
<td>1.673</td>
<td>.614</td>
<td>.448</td>
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<tr>
<td>6</td>
<td>2.073</td>
<td>2.020</td>
<td>.578</td>
<td>.385**</td>
</tr>
<tr>
<td>7</td>
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<td>2.025</td>
<td>.549</td>
<td>.353*</td>
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<tr>
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<td>2.180</td>
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<td>.414</td>
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<td>11</td>
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<td>.157</td>
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<td>.035*</td>
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<tr>
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<td>1.892</td>
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<tr>
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<td>2.119</td>
<td>.723</td>
<td>.399**</td>
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<td>1.104</td>
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<td>16</td>
<td>2.358</td>
<td>2.341</td>
<td>.098</td>
<td>.062</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Mean percentages meaningless because some rats consumed no food.

\textsuperscript{*}P<.05.

\textsuperscript{**}P<.01.

only, the differences in these means were erratic. The mean daily fecal N loss, expressed as mg/kg body wt \textsuperscript{-0.75}, was higher for Group C than for Group D on d 13 (P<.001) and 14 (P<.005) AIL in the experiment with coccidia only; and on d 6 (P<.001), 7 (P<.05), 8 (P<.025), 9 (P<.05), 11 (P<.05), 13 (P<.05), and 14 (P<.005) AIL in the experiment with the double infections (table 1). Only in the experiment with the nematodes alone was there a day (5) when the mean for Group D was significantly higher than that of Group C. The days of increased fecal N loss and decreased N absorption in the double-infected rats coincided with the period of asexual propagation of the coccidia in the cells of the intestinal epithelium. Because a similar change in the levels of fecal N did not occur in the single-infected rats, this response would suggest that effects were additive when both parasites were present simultaneously. Plasma protein leakage into the small intestine of animals parasitized by gastrointestinal nematodes has been reported (Ogilvie and Jones, 1971; Mulligan, 1972), and such loss manifested as increased fecal N could account for the present results. Symons et al. (1971), however, have demonstrated the malabsorption of amino acids in the intestines of rats infected with N. brasiliensis. In the present case, it is interesting that these differences in fecal N levels between infected and pair-fed groups were not consistently reflected in similar differences in mean N balances or in urinary: fecal N ratios. Roseby (1977) has emphasized the differences between unparasitized sheep and those with T. colubriformis in N concentration in the digesta in various regions of the intestine, as well as the resultant possible lack of reflection of these differences in fecal composition because of fermentation in the cecum and colon. Based on his research and that of others, he has constructed a scheme of N transfer between pools in infected and uninfected sheep that indicates that the rate of plasma protein N leakage into the small intestine of parasitized sheep is more than double that in unparasitized controls. Randall and Gibbs (1981) reported a reduction in apparent N absorption in calves infected with nematodes of the genera Ostertagia and Cooperia, but pair-fed control groups were not used in their investigation. In his investigation of the effects of infection by Cooperia spp. in calves, Randall (1979) found that the urinary N output was increased in clinically-infected animals even when the N intake was significantly reduced. In the present study, the mean of the urinary N as a percentage of the N consumed was significantly higher for the infected group than for the pair-fed group only in the case of the nematode infections, on d 6.
Balances of Ca and P. In the experiment with coccidia alone, the only significant difference between the daily means of Groups C and D for these elements occurred for Ca on d 8, when the Group D mean was lower (P<.01). In the experiment with nematodes only there were no significant differences in the daily mean balances of Groups C and D. With the double infections there was a significant difference between the daily means for these groups only on d 15, when the mean for Group C was higher (Ca; P<.005; P, P<.001). In no case, in any experiment there was a significant change in the proportions of Ca and P excreted in the urine and feces correlated with the presence or absence of infection.

Carcass Ca and P. The differences in mean concentrations between groups were all insignificant, indicating that the infections, under these conditions, did not produce the reductions in carcass Ca and P reported by Sykes and Coop (1976) in growing lambs infected with the intestinal nematode T. colubriformis.

Densities of Empty Carcasses. Only with the double infections was there a significant difference between the mean densities of groups. In this case, the mean density of the carcasses of Group B rats was greater (P<.005) than that of Group C or Group D rats.

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