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# Effects of Fecally Contaminated Feed by Starlings on Growing Swine & Mice

Pat Schuster *Western Kentucky University*

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Pat

# EFFECTS OF FECALLY CONTAMINATED FEED BY STARLINGS ON GROWING SWINE AND MICE

A Thesis

Presented to

the Faculty of the Department of Biology Western Kentucky University Bowling Green, Kentucky

> In Partial Fulfillment of the Requirements for the Degree Master of Science

> > by Pat Shuster December, 1979

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# EFFECTS OF FECALLY CONTAMINATED FEED BY STARLINGS ON GROWING SWINE AND MICE

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Approved Secember 19, 1979

 $m_{\pi-1}$ Dean of the Graduate College

# EFFECTS OF FECALLY CONTAMINATED FEED BY STARLINGS ON

### GROWING SWINE AND MICE

Pat Shuster December, 1979 35 pages Directed by: Herbert E. Shadowen, Joe E. Winstead and Larry N. Gleason Department of Biology Western Kentucky University

The effects of feed contamination by droppings of starlings (Sturnis vulgaris) on certain growth parameters in swine (Sus scrofa) were measured from January 25 to March 1, 1979 at the Western Kentucky University farm. Weight gain, feed-conversion efficiency and feed rejection by swine fed varying levels of contaminated feed were analyzed and found to be non-significant at the fecal concentrations used.

Laboratory mice (Mus musculus) were fed varying concentrations of starling feces in standard mouse chow; weight gain, feed-conversion efficiency and feed rejection were measured. At 0%, 10%, 25% and 50% concentrations, significant differences in weight gains at the 0.1 confidence level were found. There were no other significant differences. Caloric values of each concentration of feces in feed were also determined.

#### ACKNOWLEDGEMENTS

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#### INTRODUCTION

Aggregations of blackbirds in the winter months have resulted in many complaints from citizens who live near large roosts, ranging from the nuisance of noise and tree damage to the possibility of health hazards associated with the droppings from these birds. Farmers have also complained of economic losses resulting from the presence of birds in their livestock feeding areas and crop land. Dolbeer et al. (1978) investigated the agricultural impact of one of these roosts in Tennessee. They found that, although starlings comprised only 9% of the roosting population, they had the greatest negative impact on agriculture. This was supported by Sesser et al. (1968) who estimated that 1,000 starlings in a Colorado feedlot consumed approximately \$84 in cattle rations per year, with negligible depradations by other species. Starlings have also been implicated as possible transmitters of livestock diseases, notably Transmissable Gastro-enteritus, more commonly known as TGE, which is currently being investigated at Iowa State University under a United States Fish and Wildlife Service contract. However, most farmers complain about diminished weight gains or loss in milk production resulting from feed rejection by livestock due to the birds' fecal material and/or a possibility of reduced feed efficiency due to some inherent factor in

the feces. Thus, the Kentucky Office of Animal Damage Control, <sup>a</sup>division of the United States Fish and Wildlife Service, developed a project in order to measure feed rejection by pigs and cattle due to various levels of contamination by starlings. Weight gain and feed efficiency were to be measured under the same conditions. Starlings were chosen as the contaminators because they were implicated as the heaviest feeders in livestock areas. The project was contracted to the Department of Agriculture of Western Kentucky University which provided the barns, feed, and animals.

There is very little literature dealing with the effect on livestock of feed contaminated by starlings. Wright (1973) reported a reduction of feed efficiency in cattle due to an unspecified bird pressure level, but he did not attempt to isolate the contamination problem. However, if comparisons may be drawn between poultry and starling excreta, experiments in feeding poultry wastes as a source of dietary nitrogen have been conducted with encouraging results in light of positive weight gains. However, these experiments have dealt almost exclusively with ruminants whose mode of digestion with its large microbial population can utilize this nonprotein nitrogen. In this study the research was concerned only with swine. Although research is being conducted which deals with avian wastes fed to swine, little literature is available at this time. Perez-Aleman et al. (1971) studied growing pigs which were fed dried poultry manure and showed that for every 10% addition of manure, growth was reduced

by 0.02 kg/day and feed conversion efficiency by 0.25 units. Swine have been fed their own fecal material with no ill effects but with no enhancement of weight gains (Diggs et al. 1965). Hoefer (1967) conducted an extensive literature review concerning the effects of urea, a source of nonprotein nitrogen, as a possible protein supplement for swine. The consensus was that the monogastric swine were poorly adapted for utilization of urea and that no benefits would accrue from the addition of the material to the swine diet.

The swine experiment portion of the present research was conducted in a manner approaching normal farm practices as much as possible. As controlled conditions were limited, it was decided that starling feces would be fed to white laboratory mice under more stringent controls.

#### METHODS AND MATERIALS

The basic design of the swine study involved placing cages of starlings over the animals' feed troughs for <sup>a</sup> specified time and measuring feed rejection and weight gain by the livestock. The effects of three treatment levels or rates of bird pressure were compared to a control. The treatment levels were designated as 1X, 3X and 10X at the constant rate of 0.1 starling per 0.305 m<sup>2</sup> of trough (Fig. <sup>1</sup>and 2). These levels were calculated by personnel of the Fish and Wildlife Service using direct observations and time-lapse photography of bird activity on feed troughs. The controls were designated as OX.

The study began January 15, 1979, with the animals on treatment from January 25 to March 1 for a total of 35 days. The pigs were housed in a standard pig confinement barn, heated to approximately 20°C (Fig. 3). Water was provided by automatic waterers. They were fed a ration which consisted of ground corn and protein supplement. The ration contained 16% protein and all the vitamins and minerals necessary for optimum growth. The starlings were in chickenwire cages in the same room and were fed a commercial 16% protein ration throughout the study (Fig. 4).

Twenty-four Yorkshire-Hampshire cross-bred pigs of approximately the same age (7-9 weeks) and weights (9.53-18.16 Figure 1. Contamination process for the 1X treatment level at the rate of 0.1 starling per 0.305 m2 of trough.

Figure 2. Contamination process for the 10X treatment level at the rate of 1.0 starling per 0.305 m2 of trough.

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Figure 3. Swine confinement barn at the Western Kentucky University farm.

Figure 4. Arrangement of swine in pens with feed contamination by starlings taking place outside of pens.





kg) were randomly assigned to eight pens, three pigs per pen. This assignment was done ten days before feed contamination in order to minimize the social stress resulting from adjustment to new pen mates. There was no segregation of sexes.

The four treatment levels were replicated for a total of six pigs per treatment. Contaminated feed was available to the pigs from 7:00 A.M. to 4:00 P.M. only, again simulating normal conditions as much as possible. The nine h contamination period was also an observed figure obtained by personnel of the Fish and Wildlife Service by surveillance of the actual feeding times of starlings in feed lots. At 4:00 P.M. contaminated feed was removed, weighed with a Hansen Dairy Scale (Fig. 5) and discarded. The pigs were then fed an equal amount of fresh, uncontaminated feed which was left overnight. At 7:00 A.M. this feed was removed, weighed and discarded; contaminated feed was then placed in the troughs. The process was identical for the control treatment with the exception of bird contamination. These procedures were repeated daily during the experiment.

The contamination procedure used cages of starlings placed over troughs containing pre-weighed rations of feed. The birds were left on the troughs for nine h. The feeding troughs were 0.305 m by 1.22 m; therefore, using the rate of 0.1 starling per 0.305  $m^2$  of trough, the 1X treatment level had 1/2 bird, the 3X treatment had 1 1/2 birds and the 10X, 5 birds. The 1X and 3X treatments were accomplished by transferring the respective cages to the other troughs

Figure 5. Scale and bucket used to weigh feed.

Figure 6. Marting Single Animal Scale used to weigh swine.

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Figure 7. Animal room in the North wing of Thompson Complex, Western Kentucky University.



receiving these levels of treatment after 4 1/2 h. Each pig was identified by the ear notch system of identification and was weighed once weekly using a portable Marting Single Animal Scale (Fig. 6).

The experiments with mice were conducted in the animal room of Thompson Complex, North wing, on the campus of Western Kentucky University (Fig. 7). Twenty-four male, white mice, strain BALB/c, of approximately the same age (40 - 50 days) were randomly assigned to four cages as were 24 females, therefore eight cages in all, with six mice per cage. Sexes were kept separate in order to insure no pregnancies or sexual activity which might bias the resulting weight gains. Ample time was given for adjustment to new cage mates.

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The cages were 22.86 cm by 30.48 cm, covered with wire mesh. Water was provided by standard mouse water bottles. Bedding was also standard laboratory animal material (Fig. 8).

Starlings were housed in the animal room and maintained in the manner described for the pig study. Fecal material was collected on plastic sheets and frozen until a sufficient amount was obtained. It was then dried at 800C for 12 <sup>h</sup> and ground into a powder, using a Wiley Hammer Mill. Shannon and Brown (1969) found that there was a loss of energy and nitrogen on drying poultry excreta. Presumably, by drying the starling feces there was a similar loss; however, there was no apparent method to incorporate the fresh feces in the treated feed which would not have resulted in the mice

Figure 8. Cage with six mice, feeder and water bottle.

Figure 9. Self-feeder designed to allow mice free access to ground feed and to catch waste feed.





eating around the fecal material. Purina Pelleted Mouse Chow was also ground into a powder using a Wylie Hammer Mill. The ground fecal material and the ground chow were then mixed in the proper proportions and stored in a freezer throughout the experiment. Treatment levels were 2%, 5%, and 10% dried starling feces of the total ration of mouse chow. There was also a control in which no fecal material was added to the ground ration. Self-feeders made of No. 25 galvanized sheet metalwere designed to allow the mice to feed at liberty and to catch any feed that dropped from the feeding troughs (Fig. 9). These feeders hung in the cages at all times and were large enough to accommodate six mice feeding at one time if they so desired. As the mice fed from the spaces provided, feed would gravity-flow into the holes, therefore insuring a constantly available food supply.

Feed was weighed each day on a Sartorius Digital Precision Balance. The amount of feed actually eaten by the mice was calculated by adding the feed left to the feed wasted and subtracting the total from the amount of feed placed in the feeder the day before. The wasted feed was separated from the mice feces and bedding which had accumulated in the waste box by a sieve made from wire screen.

Each mouse was identified by a system of earpunches and was weighed every three days on the Sartorius balance mentioned above. They were on treatment for a total of 30 days from March 1 to March 30, 1979.

Three samples of each treatment ration, including untreated feed as well as the fecal material itself, were analyzed for total energy (calories) with the Parr Bomb Calorimeter.

From April 23 to May 22, 1979 the experiment was repeated using the same methodology but with an increased concentration of dried starling feces. In this study, referred to as Trial II, the treatment levels were 10%, 25% and 50%, plus a control.

#### RESULTS AND DISCUSSION

The results of this study indicate that starling feces at any concentrations approaching natural farm conditions have little, if any, negative effect on weight gain by swine. In the analysis of variance of weight gain by the pigs, there were no significant differences among the treatments (Table 1). There was a range of only 1.17 kg from the highest weight gain per treatment mean (mean weight gain of six pigs on the same treatment) to the lowest and <sup>a</sup>difference of 0.26 kg between the control and the highest level of treatment (Table 2). On examination of individual pig weight gains, the 10X group had both top-gaining pigs (27.24 and 26.33 kg, respectively) as well as the lowestgaining pig (6.36 kg). The latter animal (Pig 22-6) was ill during the experiment, and this may have accounted for the small weight gain. Whether or not the birds were instrumental in the origin of the disease will be discussed later. With such small differences in individual weight gains, except the one which was ill, these data were probably the result of chance. Whether the data were the result of chance would be difficult to ascertain without data on individual feed intake and rejection and a greater number of experimental units. As this was the first experiment examining effects of starling feces on the weight gain of swine, there existed no data with which to compare.



Table 1. Analysis of variance on average gains (kg) for swine fed four levels of fecally contaminated feed, January 25 through March 1, 1979.



Table 2. Weight gains (kg) of swine fed four levels of fecally contaminated feed, January 25 through March 1, 1979

\*Pig died before completion of experiment.

Generalizations based on such a small number of experimental units are therefore offered only on an extremely cautious basis.

The pigs did appear to select against the contaminated feed (Table 3). Animals in the pens which received the daily contaminated feed consumed a greater amount of the available uncontaminated feed. The pigs in the control pens, however, consumed more feed during the day and left more at night. This is typical of swine which will generally consume more than 50% of their total feed intake during the daylight hours. The troughs were handled identically except for the bird pressure. This selection by the pigs, although consistent throughout the experiment, involved very little feed, i.e. in 35 days the control pigs consumed approximately 5.45 kg per pen more during the day (0.045 kg/pig/day, about a large handful) whereas the experimental pigs consumed from 2.04 to 8.63 kg (average 4.99 kg) less during the day (0.045 kg/pig/day). Due to the design of the experiment it would be difficult to conclude that starling contamination would not have a detrimental effect or even a positive one on weight gain in swine if they were not allowed the choice of uncontaminated feed. The fact that the control animals rejected as much total feed as the treated animals would suggest overfeeding. This may have resulted in allowing the animals to consume less fecal contaminants by either the dilutant factor of excess feed or simply consuming more of the uncontaminated night ration. However, the paucity of





previous research dealing with these problems necessitated basic data on the swine-starling relationship under the most natural conditions possible which would allow measurements to be taken.

Feed conversion efficiency, or the number of units of feed needed to produce a unit of gain, was calculated by dividing the feed consumed by the weight gained over a 35-day period. In this study the actual amount eaten was derived by subtracting the total amount of feed rejected per pen from the amount of feed placed in the pen in 35 days. There were no significant differences among treatment feed efficiencies. These data indicate that starling feces has little effect on utilization of feed by swine, at least at the concentrations cf fecal contamination considered equivalent to feedlot situations (Table 4).

The concentration of feces to feed, even at the highest 10X level, was quite low, feces (dry weight) composing only 0.85% of the total daily (24h) feed for three pigs. When levels of 10%, 20% and 30% dried poultry manure were fed to growing swine, the resulting reduced weight gains and lowered feed efficiencies were significant only at the 20% and 30% levels (Perez-Aleman et al. 1971). Bird pressure levels used in the present experiment were obtained from actual field observation; therefore, fecal concentrations would more closely correspond to those in natural feedlot situations. Bird pressure was constant throughout the experiment; therefore, as the amount of feed increased concurrently with the increase in the growing





a<sub>Feed conversion efficiencies express kg of feed required</sub> to produce a kg of gain.

pigs' requirements, the concentration of feces decreased by <sup>a</sup>little less than one half. Again this was analogous to farm conditions in which bird pressure would be constant regardless of the amount of feed placed before the stock. The average dry weights of fecal material defecated by the starlings in each treatment level are shown in Table 5.

<sup>A</sup>further development of this study was the death of one pig and the sickness of another, both of which received the 10X level of treatment. Pig 21-3 died ten days after the beginning of the experiment. The pig which recovered had displayed similar symptoms, i.e. lack of appetite, convulsions, stiff legs and a staggering gait. After an injection of Combiotic, a broad spectrum antibiotic, it quickly recovered. The dead pig was autopsied by the Murray State University Veterinary Diagnostic and Research Center in Hopkinsville, Kentucky, but the results were ambiguous. There was 0.0006% nitrate found in the stomach contents, but this was not high enough to be considered toxic. The histopathologic diagnosis was atypical pneumonia, probably caused by Mycoplasma sp. or of viral origin. Species of Mycoplasma are common pathogens encountered in swine and other classes of livestock. In swine infection does not usually result in death; instead it is manifested by <sup>a</sup> diminished weight gain and an increased susceptibility to other respiratory infections. The stiff legs and painful movements exhibited by both pigs are symptomatic of Mycoplasma spp. infection in swine. There is some evidence that starlings



Table 5. Average weight (g) of starling feces from each bird pressure level used for contamination of swine feed, January 25 through March 1, 1979.<sup>a</sup>

a<sub>Average</sub> of seven samples.

transmit these pathogens, although formal research has not been conducted on this relationship. These pathogens are ubiquitous organisms and, being contagious, confused the issue as to why no other pigs showed any evidence of infection. This study was not intended to deal with the disease problem, although further investigations in this area are indicated.

The results of the analysis of variance of the average weight gains in mice in the first experiment, Trial I, comparing treatment levels, 2%, 5%, 10% and the control, showed no significant differences (Table 6). The results for the mice in the second experiment, Trial II, with treatment levels of 10%, 25%, 50% and the control did not show a significant difference among treatment levels at the 0.05 confidence level (Table 7). However, at the 0.1 level there was a significant difference, indicating that at higher concentrations of fecal material, significant effects on weight gains are more pronounced. All mice on the 50% treatment lost weight. The males showed the most dramatic decrease. By May 4, 12 days into the experiment, males had lost from 19% to 40% (mean 30%) of their original body weight. At that point death and cannibalism resulted in one mouse being partially eaten. Two more mice were totally eaten with the exception of the hair and tails two days later. As any further data on weight gains for the survivors would be biased, they were placed on rations containing no feces. All calculations on this group were based on the

	Control		2 <sub>8</sub>			
<b>Replication 1</b>		<b>Replication 2</b>	Replication 1	<b>Replication 2</b>		
5.282		4.527	2.662	4.203		
	5%		10%			
Replication 1		<b>Replication 2</b>	<b>Replication 1</b>	<b>Replication 2</b>		
2.987		3.180	2.507	3.987		
Source	df	SS	<b>MS</b>	$\mathbf{F}$		
<b>Total</b>	7	6.792				
Replication	$\mathbf{1}$	0.756	0.756	1.239 N.S.		
<b>Treatment</b>	3	4.206	1.402	2.298 N.S.		
Error	$\overline{\mathbf{3}}$	1.830	0.610			

Table 6. Analysis of variance of average weight gain (g) for mice fed four levels of fecally contaminated feed, February 28 through March 30, 1979, Trial I.

	Control		10%			
<b>Replication 1</b>		<b>Replication 2</b>	<b>Replication 1</b>	<b>Replication 2</b>		
4.00 3.11			1.44	2.295		
25%			50%			
<b>Replication 1</b>		<b>Replication 2</b>	<b>Replication 1</b>	<b>Replication 2</b>		
3.51	0.925		$-6.08a$	$-2.998$		
Source	df	SS	MS	E		
Total	7	87.27				
Replications	$\mathbf{1}$	0.03	0.027	0.009 N.S.		
<b>Treatments</b>	3	78.40	26.13	8.858 N.S.		
Error	3	8.85	2.95			

Table 7. Analysis of variance of average weight gains (g) for mice fed four levels of fecally contaminated feed, April 23 through May 22, 1979, Trial II.

aWeight loss for 12 days.

first 12 days of the experiment. All females on the 50% treatment survived. At the end of the 30 days, weight loss ranged from 0.2% to 29% with the mean and the mode being 18% of the orignal body weights. The individual which showed the 0.2% loss was unique in that it had lost 18% of its original body weight by May 1, nine days into the experiment, then demonstrated a fairly regular increase in weight gain until the termination of the study. The differences between male and female mice treated at the 50% level may have occurred as a result of a greater adaptability of females, <sup>a</sup>greater tolerance for some substance in the fecal material, or some other factor, but studies of this nature were beyond the scope of this study. Also, whether or not there would be consistent differences by males and females to this fecal concentration would require more replications and experimental units.

It was expected that if palatability were the most important factor involved in feed rejection and thus a factor in weight loss, a pattern of increased wasted feed would emerge as concentrations increased. With one exception this pattern occurred (Table 8). There was generally greater wastage in Trial II, and males wasted more than females in every grouping except for those at the 25% treatment level. Palatability probably was responsible for the general decrease in feed eaten as the concentration of feces increased (Table 9). This rejection of feed because of apparent lack of palatibility was reflected by the fact that the mice





27.



Table 9. Feed conversion efficiencies (g) per cages on different treatment levels for 30 days, Trials I and II.<sup>a</sup>

<sup>a</sup>Feed conversion efficiencies express g of feed required to produce a g of gain.

bAmount eaten in 12 days.

cFeed efficiencies not calculated for weight loss.

on the 50% level showed a marked increase in weight (males - 3.24 g and females - 5.70 g) after a week on uncontaminated feed.

Feed conversion efficiency was calculated in the same manner as the swine, i.e. on a 30-day basis the total feed eaten was divided by the total weight gain of six mice per cage of a treatment level (Table 9). There was a general reduction in feed efficiencies in Trial II when compared to Trial I which may be attributed to several factors. The room in which the mice were housed was sightly warmer and more humid during the second trial which may have resulted in lodging of the ground feed which would have interfered with the free flow of feed into the access holes. Therefore, the mice would have had less food available and possibly more competition would have arisen. A combination of these factors affecting the mice in Trial II would correspond with the over-all pattern of smaller weight gains in the second trial.

Another possible factor to be considered in the discrepancies of the feed efficiencies between trials could have been the starlings. The increase in the photoperiod, hormonal changes may have altered the composition of the fecal material in some way as to influence its digestibility.

At higher concentrations of fecal material there appeared to be a reduced, although not always consistent, feed efficiency. Monogastric animals with their non-protein nitrogen utilizers, i.e. intestinal microorganisms, concentrated in the lower digestive tract have difficulty dealing with high levels of

nitrogenous material (Hoefer, 1967). Possibly, stress resulting from physiological attempts to handle the extraneous fecal material may have caused lower feed efficiencies. Also, nitrates present at a slightly toxic level may have lowered them.

Caloric values were determined in order to ascertain whether any weight loss while on contaminated feed could be attributed to a difference in the energy value resulting from the addition of the feces. The differences were small and not significant (Table 10). The correlation between calories per day and average weight gain per day in Trial I was 0.001, showing essentially no relation. In Trial II there was an increase in the correlation  $(r = 0.535)$ . This can be expressed by stating that 28.6% of the variation of weight gain may be attributable to the daily caloric intake of the mice (Table 11). A correlation of 0.535 is not considered to be high, but this statistical method when applied to these data would involve multiplying calories per day by gain per day, placing an emphasis on the total mount of feed eaten. The 50% group in Trial II ate less than half the amount consumed by the other groups, which may have skewed the correlation higher than it would have been had that group eaten similar amounts of feed.



Table 10. Energy levels (calories) of treatment materials fed to mice: Trial I, March 1 - 30, 1979 and Trial II, April 23 - May 22, 1979

aAverage of three samples each.

<b>Treatment Cage</b>		Calories (c)	Eaten/ $day$ $(g)$	(X) Calories/ Gain/ day(c)	(Y) $day$ $(g)$	(XY)
			Trial I			
0%	M	$\sim$ 48 4287	3.783	16217.7	0.176	2854.3
	F		3.100	13289.7	0.151	2006.7
28	M	4210	3.355	14124.6	0.089	1257.1
	F		2.997	12617.4	0.140	1766.4
5%	M	4255	3.475	14784.0	0.099	1463.6
	F		2.605	11084.3	0.106	1174.9
10%	M	4236	3.137	13288.3	0.084	1116.2
	F		2.843	12042.9 107448.0	0.133 0.978	1601.7 13240.9
			Trial II			
0%	M	4188	3.755	15725.9	0.133	2091.5
	${\bf F}$		3.230	13527.2	0.104	1406.8
10%	M	4074	3.256	13264.9	0.048	636.7
	F		3.045	12405.3	0.077	955.2
25%	M	4102	3.443	14123.2	0.117	1652.4
	F		2.603	10677.5	0.031	331.0
50%	M	3926	1.292	5072.4	$-0.501$	$-2541.3$
	F		1.626	6383.7 91180.I	$-0.100$ $-0.091$	$-638.4$ 3893.9
Trial $I - r = 0.001$						

Table 11. Correlation between average daily weight gains and daily caloric intakes of mice in Trials <sup>I</sup> and II.

Trial II - <sup>r</sup><sup>=</sup> 0.535

#### SUMMARY

The effects of feed contamination by starling feces on weight gains and feed efficiencies in swine were not significant, at least at the concentrations considered equivalent to feedlot situations. These results may have been biased as the swine had access to uncontaminated feed at night and did appear to select against the contaminated feed, although the amount per pig per day was small. Thus, the amount of fecal material actually ingested by the pigs was unknown. However, a tentative conclusion may be offered that fecal material of starlings was not directly harmful to the swine's growth. The economic loss resulting from reduced growth rates in swine would appear to be minimal. With the proliferation of enclosed swine barns and self-feeders with hinged covers if the swine are maintained outdoors, the swine-starling problem would seem to be overrated. The issue of disease transmission by the presence of the birds or their droppings appeared as one pig died and another was ill. This problem should be investigated more thoroughly.

The mice in Trial I showed no significant differences in weight gains or feed efficiencies. The weight gains for mice in Trial II approached statistical significance. Feed efficiencies were generally reduced as the fecal concentrations increased. It would appear that at higher concentrations of

fecal material, growth in mice was reduced because of reduced feed consumption.

Caloric contents of each treatment ration were determined in order to examine the possible energy dilutant factors of fecal additions. These were found to be insignificant.

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