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## THE INFLUENCE OF FEEDING LACTOSE OR DRY SKIM MILK ON ARTIFICIAL INFECTION OF CHICKS WITH EIMERIA AVIUM

J. R. BEACH AND D. E. DAVIS

#### INTRODUCTION

The experiments reported in this paper consist of a series of five trials in which it was attempted to combat artificially-produced coccidial infection in chicks by feeding them with sufficient lactose or dry skim milk to change the hydrogen ion concentration of the ceca from the normal range of 6.0-7.4 to a range of 4.4-5.6. It was thought that, by this means, an environment unfavorable or destructive to the tissue-invading stages of the parasite, viz., the sporozoites and merozoites, might be created.

The first three trials were carried out under laboratory conditions, the chicks being confined in cages with grilled bottoms and fed in cups suspended on the cage doors. In the last two trials, the chicks were reared in brooder pens under normal field conditions, except that no outside runs were provided.

After the feeding of lactose or dry skim milk was begun, the chicks were inoculated by introducing into their crops with a pipette a large number of sporulated oöcysts of *Eimeria avium*. A control group of chicks that was fed neither lactose nor dry skim milk was included in each trial. An estimate of the number of cysts administered to each chick was obtained by making a direct microscopic count of the cysts in  $\frac{1}{100}$  c.c. of the inoculum. Material for inoculation was provided by cultures of the cecal contents of chicks affected with coccidiosis prepared as follows: A thin layer of cecal contents containing large numbers of oöcysts was spread over the surface of salt solution agar plates.<sup>1</sup> Salt solution to keep the surface of the plates moist was added as required. The cultures were incubated at room temperature until microscopic examination showed that sporulation of the oöcysts had occurred.

<sup>&</sup>lt;sup>1</sup> The writers are indebted to H. W. Graybill for suggesting the use of and furnishing the formula for the "salt solution agar." The formula is as follows: Agar, 20 gms.; sodium chloride, 5 gms.; distilled water, 1000 c.c. The agar is cut up, tied in a gauze bag and washed for two hours in running water before the medium is made up.

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21	P.M.	5.6	١	1	1	5.4	В		А		В
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19	P.M.	5.6		5.0	1	I	1	6.0		5.4	I
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17	P.M.		1	1	1	I		1	I	1	
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. 16	P.M.	1	1	١	1	I	1	1	I	1	I
Mar. 16	A.M.	1	5.6	I	I	5.4	5.6	6,2	6.0	6.6	I
. 15	P.M.	5.6	1	4.8	4.8	5.2	6.0	5.8	I	I	
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Fowl	No.	51	52	53	54	55	56	57	58	59	09
				ojə Inc					110 dno		

TABLE 1

PH OF CECAL DROPPINGS AND EFFECT OF INOCULATION WITH SPORULATED OÜCYSTS IN FIRST TRIAL

S=Bird visibly sick.

B=Cecal droppings contain blood.
 D=Died from coccidiosis.
 X=Cecal droppings watery and mixed with other portion of droppings.

--=No cecal droppings:

#### FIRST TRIAL

Ten chicks, four weeks old, were divided into two groups of five. They had been reared in an environment thought to be free from *Eimeria avium*. From March 14, 1924, each bird in Group I was given 1 gram of lactose twice daily at 9 a. m. and 4:30 p. m. Group II, the control, received no lactose. On March 15, approximately 45,000 sporulated oöcysts were introduced into the crop of each bird in both groups. Cecal droppings for pH determinations were collected twice each day. The pH determinations of the cecal droppings and the effect of the inoculation on the chicks are recorded in table 1.

The pH determinations of the cecal droppings showed a constant higher degree of acidity in the ceca of the birds of the lactose group than in the controls.

Blood appeared in the droppings of two birds (nos. 52 and 54) of the lactose group on the sixth day. No. 52 passed blood for one day only, but No. 54 continued to do so for three days. Merozoites were present in the bloody droppings from these birds. None of the birds in this group were otherwise visibly affected. Oöcysts were found in the droppings of all birds after the sixth day.

Three of the five controls were passing bloody droppings on the fifth day. All passed blood and three died from coccidiosis on the sixth day. The fourth death from this cause occurred on the ninth day. The one remaining bird ceased passing blood after three days and exhibited no further symptoms.

All the birds were killed for autopsy on the eleventh day. The ceca of four of those in the lactose group appeared to be normal. One cecum of No. 52, was filled with a caseous core.

Both ceca of the one survivor of the control group were filled with a bloody, caseous core.

The results indicate that the lactose feeding was of marked benefit in combatting artificial infection with sporulated oöcysts of *Eimeria avium*. Two of the birds in the lactose group passed bloody droppings in which merozoites were present, and oöcysts occurred in the cecal droppings of all birds. This is evidence that at least a part of the sporozoites released from the sporocysts were unharmed and invaded the cells of the cecal mucosa where both the sexual and asexual cycles of development were completed. It is possible that the dose of oöcysts was too large to be entirely overcome or that the increased acidity in the ceca was more destructive to the merozoites than to the sporozoites.

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ABLE	

PH OF CECAL DROPPINGS AND EFFECT OF INOCULATION WITH SPORULATED OUCYSTS IN THE SECOND TRIAL

Apr.8	A.M.	x	X	X		x			**S		s D*
Apr. 7	P.M.			1		1					
dΡ	A.M.	B	В	В		в			s		s
Apr. 6	P.M. A.M.	B	I	в	1	в			$\mathbf{B}$ S	D	$\mathbf{B}$ S
чb	A.M.	B	В	B	р	В		n A	в	$\mathbf{B} \mathbf{S}$	$\mathbf{B} \mathbf{S}$
Apr. 5	A.M. P.M.		Х	х	Х	I					I
чрі	A.M.	5.6	5.0	5.4	I	x	B S B	a a B	В	$\mathbf{B} \mathbf{S}$	$\mathbf{B}$ S
4	P.M.	5.4	5.4	5.6	×	5.2	L H	-	В	В	
Apr. 4	A.M.	5.4	5.2	5.4	5.0	5.2	6.0	6.0	1	6.4	5.6
~~.	P.M.	5.2	5.4	5.4	1	5.0	6.0	6.2	I	1	
Apr. 3	A.M.	5.6	5.4	5.4	5.2	1	6 4	8.9	6.0	6.0	6.4
. 2	P.M.	5.0	5.4	5.2	I	5.2	1	1	1	I	I
Apr.	A.M.	5.2	5.0	5.6	5.4	5.2	99	6.6	I	6.0	6.6
	P.M.	5.4		5.6	x	5.0		1	6.4	I	6.4
Apr. 1	A.M.	5.2	5.4	5.2	x	5.4	6.2		6.6	6.6	6.4
. 31	P.M.	x	5.0	5.2	x	x			1	5.6	1
Mar.	A.M.	5.4	5.0	5.2	5.4	5.2	5 4	6.6	5.8	6.0	6.0
. 30	A.M. P.M.	x	x	x	×	x		1	ł	I	1
Mar.	A.M.	5.4	5.2	5.2	5.4	5.0	6.6		6.2	I	6.2
r. 29	P.M.	5.2	5.2	5.4	5.0	5.2		1	I	1	6.2
Mar.	A.M	5.6	5.4	5.2	5.0	5.2	6.4	6.8	6.8	6.6	6.2
	Bird No.	61	62	63	64	65	99	67	89	69	20
			I q			)		II (slo			

S=Bird visibly sick.

B = Cecal droppings contain blood. D = Died from coccidiosis. X = Cecal droppings watery and mixed with other droppings. = No cecal droppings. = No. 70 diad on Apr. 12. \*= No. 68 recovered.

#### SECOND TRIAL

Ten chicks, six weeks old, were divided into two groups of five. The method of procedure was the same as in the first trial. The feeding of lactose to the birds in Group I was started on March 28, 1924. Three days later, approximately 40,000 sporulated oöcysts were introduced into the crop of each bird of both groups. The pH determinations of the cecal droppings and observations on the effect of the inoculation are given in table 2.

As in preceding experiments, an abnormal degree of acidity in the ceca was produced by the feeding of lactose.

One bird of the lactose group began voiding bloody droppings on the fifth day and three others on the sixth day. Both merozoites and oöcysts were found in the droppings. The appearance of the droppings had become normal on the eighth day and these birds exhibited no further symptoms. The fifth bird of Group I was found dead from coccidiosis on the morning of the sixth day. It had not previously appeared sick nor passed blood with the droppings.

Three of the control group were voiding bloody droppings on the fourth day. On the fifth day all showed marked droopiness and were passing blood. Four died from coccidiosis, three on the sixth day and one on the twelfth day. The remaining bird was visibly sick for several days but finally recovered in so far as the manifestation of symptoms was concerned.

All survivors were killed for autopsy on the fifteenth day after inoculation. The ceca and other organs of the four birds of the lactose group were normal in appearance. Microscopic examination of the cecal contents for coccidial cysts was negative.

The ceca of the one survivor of the control group were found to be entirely filled with solid, bloody, caseous cores which contained numerous oöcysts.

These results closely parallel those of the preceding trial. It was again demonstrated that feeding lactose did not prevent the sporozoites from invading the epithelial lining of the ceca where the cycles of development of the parasites were completed. In four of the five birds, however, further development of disease was arrested. The death of the one bird might have resulted entirely from the tissue damage caused by the invasion of the sporozoites and completion of their developmental cycles. This would appear to be additional evidence that the acidity produced in the ceca by feeding lactose to chickens is more destructive to the merozoite than to the sporozoite forms of *Eimeria avium*.

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TABLE 3	BIRDS
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fed         ber of birds         A.M.         P.M.         P.M.         P.M.         A.M.         P.M.	No.         fed         brevol         AM         PM         <		Care Ma	Mash Num-		May 21	Ma	May 22	May	53	May 24	24	Ma	May 25	Ma	May 26	Ma	May 27	May 28	7 28	Ma	May 29
(I)       I)       I) <t< th=""><th>(Ioninoo)       Induction       Induction       Induction         (Ioninoo)       Induction       Induction       Induction         Induction       Induction       Induction       Induction</th><th></th><th></th><th>i ber of birds</th><th></th><th></th><th></th><th>P.M.</th><th></th><th></th><th></th><th>P.M.</th><th>A.M.</th><th>P.M.</th><th>A.M.</th><th>P.M.</th><th>A.M.</th><th>P.M.</th><th>A.M.</th><th>P.M.</th><th>A.M.</th><th>P.M.</th></t<>	(Ioninoo)       Induction       Induction       Induction         (Ioninoo)       Induction       Induction       Induction         Induction       Induction       Induction       Induction			i ber of birds				P.M.				P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
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Omega       Allower	Mood         Mouth	to D		_	7.4		7.2	7.0	6.8	6.8	6.8		в	в	в	1	No cet	al dropp	ings pass	ed after	May 26.	
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13       13         15       5.4       5.2       5.6       5.2       5.1         14       5.6       5.2       5.6       5.2       5.7       5.6         11       5.4       5.2       5.6       5.2       5.6       5.2       5.7         15       5.4       5.0       5.4       5.2       5.6       5.2       5.7         11       5.4       5.0       5.4       5.8       5.2       5.0       5.4       5.9         11       5.4       5.2       5.6       5.2       5.0       5.4       5.9       5.2       5.1         11       5.4       5.4       5.4       5.8       5.6       5.2       5.1       5.2       5.1       5.2       5.1       5.2       5.1       5.2       5.1       5.2       5.1       5.2       5.1       5.2       5.1       5.2       5.1       5.2       5.1       5.2       5.2       5.1       5.2       5.2       5.1       5.2       5.2       5.2       5.2       5.2       5.2       5.2       5.2       5.2       5.2       5.2       5.2       5.2       5.2       5.2       5.2       5.2       5.2	B=Dronoines contain blood					×		5.4	5.0	5.4		I oV	5.0 B	I	B	1	I	1	1		5.4	1
13       1       5.4       5.2       5.6       5.2       5.0       5.0       5.1       5.0       5.1       5.0       5.1       5.0       5.1       5.0       5.2       5.2       5.2       5.0       5.1       5.0       5.2       5.2       5.0       5.1       5.0       5.1       5.0       5.1       5.0       5.2       5.0<	13         1           16         1           16         6.6           17         7           17         8           20% Lactose Mash         7           17         8           18         5.6           18         5.6           11         7           12         4           13         7           14         7           15         4           16         5.6           17         7           18         7           11         7           12         1           13         5.6           5.6         5.7           16         5.6           17         7           18         5.6           5.6         5.7           7         5.6           5.6         5.7           7         5.6           7         5.6           7         5.6           7         5.6           7         5.7           8         5.6           7         5.7           7											I										
16       16       5.4       5.0       5.4       5.0       5.4       5.2         11       14       5.6       5.4       5.0       5.4       5.2       5.4       5.2         11       17       5.4       5.2       5.4       5.2       5.4       5.2       5.2         11       17       5.4       5.2       5.4       5.2       5.4       5.2         0%       1.sectosee Massi       5.6       5.4       5.2       5.4       5.2       5.2         0%       1.sectosee Massi       5.0       5.4       5.2       5.4       5.2       5.2       5.4       1       1       1         17       5.0       5.2       5.4       5.8       5.6       1 <td>16         14         6         5           14         14         6         6         5           17         17         4         5         4         X           15         20% Lactose Mash         4         5         4         X         5           18         20% Lactose Mash         4         5         6         5         5           18         1         X         4         5         6         5         5           18         1         X         5         6         5         6         5         5           18         1         X         5         6         5         6         5         6         5         6         5         6         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5</td> <td></td> <td></td> <td>-</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>5.2</td> <td></td> <td>5.0</td> <td>1</td> <td>B</td> <td>   </td> <td>5.2 B</td> <td>1</td> <td>1</td> <td>I</td> <td>5.2</td> <td>5.0</td>	16         14         6         5           14         14         6         6         5           17         17         4         5         4         X           15         20% Lactose Mash         4         5         4         X         5           18         20% Lactose Mash         4         5         6         5         5           18         1         X         4         5         6         5         5           18         1         X         5         6         5         6         5         5           18         1         X         5         6         5         6         5         6         5         6         5         6         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5         5         6         5			-							5.2		5.0	1	B		5.2 B	1	1	I	5.2	5.0
14       14       14       14       14       14       14       14       14       14       14       14       15       17       17       17       17       16       17       1	14         8         4         5.4         X           17         17         4         5.4         X           15         20% Lactose         4         5.4         X           18         20% I         4         5.6         5.           B=Drombines contain blood.         1         X         5.6         5.				6.6	5.	5.4	5.0	5.0	5.4	5.2		5.4 B	I	B	1	5.2 B	1	1	1	5.2	5.0
14       5.4       5.4       5.8       5.6B       1       1         17       4       X       5.2       5.4       5.8       5.6B       1       1         17       4       X       5.2       5.4       5.8       5.6B       1       1         17       4       X       5.2       5.0       5.0B       1       1       1         16       5.6       5.0       5.2       5.0       5.0B       1       1       1         16       5.6       5.0       5.2       5.0       5.0B       1       1       1         16       5.6       5.0       5.4       5.8       5.0B       1       1       1         17       1 <td>14         16         4         5.4         X           17         16         266         4         X         5         5           18         30%         4         5.6         5         6         5           18         30         1         X         5         6         5         5           8=         Dromines contain blood.         1         X         5         5         5         5         5</td> <td>II</td> <td> M a</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>5.4</td> <td></td> <td>6</td> <td></td> <td>f</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>с 1</td> <td></td>	14         16         4         5.4         X           17         16         266         4         X         5         5           18         30%         4         5.6         5         6         5           18         30         1         X         5         6         5         5           8=         Dromines contain blood.         1         X         5         5         5         5         5	II	 M a								5.4		6		f						с 1	
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15 15 16 5.4 7.0 5.0 7.0 5.4 5.8 5.0B B	15         1         4         5.6         5.           18         20         4         5.6         5.           B= Dronbings contain blood.         3         3         3         3	noı		-	×	2.5	5.4		5.0	5.2	5.0 1		9.0 B	I	>	1	0.0	1	1		4.0	
	B     R     1     X     5.4     5.2     5.2     5.2     5.4     B     All birds dead.     1     1       B     Droppings contain blood. When "B" alone appears in a space, it indicates that all cecal droppings contained blood and no normal samp     B     Droppings contained blood and no normal samp	Ð			5.6	10	7 0	20	2 0		0 00 # 10		5.0 B		В	1	١	1	1	1	5.2	1
×   1   X   5.4   5.2   5.2   5.2   5.2   5.4   B							5.2	5.2	5.2				В		All bir	ds dead.						

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#### THIRD TRIAL

In this experiment, the protection of chicks against coccidiosis was attempted by feeding them lactose or dry skim milk mixed with their mash. Sixty-eight chicks, six weeks old, were used. On May 20, approximately 50,000 sporulated oöcysts were introduced into the crop of each bird. The chicks were divided into three groups and immedately supplied wth the following rations:

Group I (controls), consisting of twenty-four birds, was fed a plain mash mixture.

Group II, consisting of twenty-two birds, was fed mash containing 40 per cent dry skim milk.

Group III, consisting of twenty-two birds, was fed mash containing 20 per cent lactose.

The dry skim milk contained 50.6 per cent lactose. This made the lactose content of the mashes for groups II and III approximately the same.

Fourteen deaths from chilling occurred during the three days following infection. The number of birds in Group I was thus reduced to twenty and in Groups II and III to seventeen each.

Mash was kept constantly before the birds, no other food being supplied. No preliminary feeding of dry skim milk or lactose before the administration of oöcysts was given.

From two to four birds were placed in each cage. Samples of cecal droppings for pH determinations were collected twice daily. Since there was more than one bird in a cage, it could not be determined from which individual a particular sample originated. This procedure, however, served to show differences between the pH values of cecal droppings of the three groups. When all deposits of cecal droppings from the birds in a cage were of the same physical character, one sample only was taken, otherwise more than one sample was taken. The first samples of droppings were collected on May 21, the day after the birds were inoculated and the feeding of lactose and dry skim milk was begun. The pH determinations and a summary of the effect of the inoculation on the birds are given in tables 3 and 4.

With few exceptions, the pH of the cecal droppings from the birds of Groups II and III was between 4.8 and 5.6, while in Group I, it ranged from 6.2 to 7.4.

A portion of the cecal droppings passed by Groups II and III on the fifth day contained some blood. This was more marked on the sixth day. A slight amount of blood was present in the droppings on the seventh and eighth days, but thereafter they were normal.

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Group No.	Mash fed	Num- ber of birds		6th day	7th day	8th day	9th day	Total died from coccidiosis	
I	Plain	20	4 B	4 B	x	x	x	18	90
Controls			88	8 S	2 S	2 S	08		
			3 D	8 D	6 D	1 D	0 D		
II	40%	17	2 B	3 B	2 B	1 B	0 B	10	58.8
	Dry skim		1 S	4 S	08	08	0 8		ĺ
	milk		2 D	6 D	2 D	0 D	0 D		
III	20%	17	2 B	3 B	2 B	0 B	0 B	11	64.7
	Lactose		$2 \mathrm{S}$	$2 \mathrm{S}$	08	0 S	0 S		
			3 D	7 D	1 D	0 D	0 D		

#### TABLE 4

EFFECT OF INOCULATION WITH SPORULATED OCCYSTS IN THE THIRD TRIAL

D=Died from coccidiosis. Numeral preceding indicates number of birds.

S=General symptoms such as droopiness, inappetence. Numeral preceding indicates number of birds.

1 B=Blood present in less than half of cecal droppings.

2 B=Blood present in more than half of cecal droppings, but not in all.

3 B=Blood present in all cecal droppings, but all droppings not entirely blood.

4 B=Cecal droppings appear to be entirely blood.

X = No cecal droppings passed.

\*=No indications of coccidiosis before fifth day.

Blood was discharged profusely from all birds in Group I on the fifth and sixth days. No cecal droppings were passed by these birds after the sixth day.

As shown in table 4, the first deaths from coccidiosis in all three groups occurred on the fifth day. The total mortality from this cause was eighteen, or 90 per cent, in Group I; ten, or 58.8 per cent, in Group II; and eleven, or 64.7 per cent, in Group III.

Besides suffering a lower mortality than the controls, a smaller number of the birds that were fed dry skim milk or lactose exhibited general symptoms and their droppings contained less blood.

The mortality from coccidiosis was relatively high in all groups. This may have been influenced by a chilling the birds received during the first two nights after the experiment was begun and also by the large dose of oöcysts they received. The results demonstrate, however, that feeding chicks mash containing 40 per cent dry skim milk, or 20 per cent lactose, is of considerable benefit in protecting them against artificial infection with sporulated oöcysts of *Eimeria avium*.

#### FOURTH TRIAL

This experiment was designed to show the value of feeding of dry skim milk or lactose in protecting chicks, kept under conditions approximating those found in the field, against artificial infection with sporulated occysts of *Eimeria avium*.

Day-old chicks from a commercial hatchery were transferred directly to clean brooder pens. To avoid natural infection with coccidiosis, no outside runs were provided.

The chicks were divided into three groups of two pens each. To Group I was fed the following mash mixture:

The mash mixture for Group II consisted of:         Lactose       20 parts         Wheat bran       30 parts
-
Wheat bran 30 parts
Bone meal 10 parts
Meat scrap 15 parts
Ground barley 15 parts
Yellow corn meal 10 parts
Cod-liver oil 2 parts
For Group III, the controls, the mash was:
Wheat bran
Bone meal
Meat scrap 15 parts
Yellow corn meal 30 parts
Ground barley
Cod-liver oil

At the time these experiments were in progress there was no supply of green feed available in our location which was known to be free from contamination with oöcysts, therefore, cod-liver oil was included in the mash to supply vitamin A.

Scratch grain fed to all pens consisted of equal parts of fine cracked yellow corn, steel cut oats, and cracked wheat.

The mash and grain were fed in the proportion of two parts of mash to one part of grain. Fed in this proportion, the nutritive ratio of the

	Per cent	42.1 77.7 92.	None 21.7 4.1
	Total	8 14 23	None 5 1
liosis	6th day 7th day 8th day 12th day 13th day* Total	None None None	None None 1
Died from coccidiosis	12th day	None None 1	None None None
Died	8th day	2 2 None	None 1 None
	7th day	4 2 9	None 3 None
	6th day	2 7 16	None 1 None
Method of inoculation		250,000 oocysts orally	Milk powder         12,500,000 oocysts in soil           Lactose         12,500,000 oocysts in soil           Plain (control)         12,500,000 oocysts in soil
Mash fed		Milk powder Lactose Plain (control)	Milk powder Lactose Plain (control)
Number of chicks	March 5	19 18 25	24 23 24
Pen No.			~ ~ ~
Groun	No.	тщ	

\* No deaths occurred after the thirteenth day.

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TABULATED SUMMARY OF FOURTH TRIAL

TABLE 5

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rations for all groups was 1 to 3.0.\* Mash in metal hoppers was kept before the chicks continuously. The amount of mash consumed was determined by placing a weighed amount in the hoppers each morning and weighing out the unconsumed portion on the following morning. The difference in the weights represented the amount consumed during the preceding twenty-four-hour period and served as an index of the amount of grain to feed to preserve the two to one mash and grain ratio.

When the chicks were fourteen days old, inoculations with sporulated oöcysts were made as follows: Approximately 250,000 oöcysts were introduced into the crop of each chick in Pen I of all three groups. In Pen 2 of all three groups was placed a box of sterilized soil to which was added approximately 12,500,000 oöcysts. The grain fed in these pens thereafter was scattered on the soil. It was thought that by this means the chicks could be made to acquire coccidial infection in a more natural manner.

At the time the oöcysts were administered, the number of chicks in the different pens was as follows:

Group I-Pen 1 contained 19 chicks; Pen 2, 24 chicks

Group II-Pen 1 contained 18 chicks; Pen 2, 23 chicks

Group III-Pen 1 contained 25 chicks; Pen 2, 24 chicks

The oöcysts were administered on March 5. The chicks were kept under observation until March 31.

Deaths occurred from coccidiosis on the sixth day after inoculation and continued until March 18, the thirteenth day. There was no sickness nor death after that date. A tabulated summary of results is given in table 5.

In this table, it is seen that the total mortality of birds receiving the oral administration of oöcysts was 92 per cent in the control pen, 77.7 per cent in the lactose pen, and 42.1 per cent in the dry skim milk pen. Such relatively high mortality in all pens is not greater than is to be expected in view of the enormous dose of oöcysts given to each chick. The results demonstrate, however, that feeding chicks mash containing 40 per cent of dry skim milk affords them considerable protection against severe coccidial infection.

The results obtained with lactose were much less satisfactory. The results of preceding experiments indicate that the value of dry skim milk for coccidiosis control lies in the lactose it contains. Therefore, mashes containing 20 per cent lactose and 40 per cent dry skim

<sup>\*</sup> The writers are indebted to W. E. Newlon for assistance in compounding the rations.

milk should be equally effective. A possible explanation of the failure of lactose mash in this case is that a sufficient quantity was not consumed. It was observed that much of this mash was scattered in the litter about the feed hopper, while the dry skim milk mash was consumed without waste.

The difference in the character of the two mashes appeared to be the factor responsible for the waste in one case and lack of waste in the other. The lactose mash contained 30 per cent of bran and 15 per cent of meat scrap. This made a coarse, flaky mixture that was easily scratched out of the hopper. The coarse brown particles of meat scrap appeared to be attractive to the chicks and in their efforts to pick them out the lighter part of the mash was thrown out on the floor and lost. The dry skim milk mash, on the other hand, was a uniform, nearly white, somewhat adhesive mixture of fine texture which did not tempt the chicks to pick through it and which was, therefore, consumed without waste.

The only serious mortality resulting from feeding grain on soil with which oöcysts had been mixed was that of 21.7 per cent in the lactose pen. No chicks died in the dry skim milk pen and only 4.1 per cent died in the control pen. The explanation of the slight degree of infection among the control chicks is evidently the failure on their part to ingest enough oöcysts to produce disease, since it is definitely known that the oöcysts were present in the soil and that the chicks were susceptible.

#### FIFTH TRIAL

The method of procedure followed in this experiment, with the exception of the changes noted below, was the same as in the immedately preceding one. Pens in which infecton was attempted by means of feeding grain in soil contaminated with sporulated oöcysts were omitted because of the uncertainty of infecting chicks by this method. The mash mixtures were the same, except that wheat shorts were substituted for wheat bran and the meat scrap was sifted through a fine screen. The purpose of these changes was to provide as nearly as possible the same degree of fineness in all mashes. It was thought that by this means the temptation for the chicks to pick over the mash would be removed and the wasting of mash from this cause thereby avoided.

One hundred and fifty chicks, forty-eight hours old, were divided into three pens of fifty chicks each. They were given their first feed when seventy-two hours old. Pen 1 received the 40 per cent skim milk powder mash; pen 2, the 20 per cent lactose mash, and pen 3, the controls, the plain mash.

During the first five days, the ration consisted entirely of mash which was before the chicks at all times. At this point, the sudden onset of a period of cold, damp weather had an unfavorable effect on all of the chicks, but was more serious among those in Pens 1 and 2 than in the control pen. This appeared to be due to the fact that the litter in Pens 1 and 2 became damp, while that in Pen 3 remained drv. This dampness resulted from the watery consistency of the droppings from the chicks fed dry skim milk and lactose. It was thought desirable, therefore, to reduce consumption of these mashes until the weather moderated by feeding scratch grain in addition to the mash. Scratch grain was fed twice daily for ten days. The chicks in all pens now appeared equally vigorous. From this time until the termination of the experiment, grain was fed in the morning only and the amount supplied restricted to one-third that of the mash consumed.

On April 30, when the chicks were eighteen days old, 1000 sporulated oöcysts were introduced into the crop of each chick. Pen 1 now contained forty-two chicks; Pen 2, thirty-nine chicks; and Pen 3, forty-two chicks.

Deaths from coccidiosis began on the sixth day after inoculation and continued through the seventh and eighth days.

A summary of the results is given in table 6:

Pen No.	Ration	Number of chicks inoculated	Total died from coccidiosis	Per cent died from coccidiosis	Average weight per chick 32 days old	Average daily mash consump- tion per chick
1	Skim milk powder mash	42	1	2.3	185.5 gm.	12.5 gm.
2	Lactose mash	39	3	7.7	133.3 gm.	10.9 gm.
3	Plain mash (control)	42	10	23.8	146.9 gm.	12.9 gm.

TABLE 6 TABULATED SUMMARY OF FIFTH TRIAL

The results, as shown in this table, clearly demonstrate the effectiveness of dry skim milk in combatting coccidial infection. Bloody droppings were passed by several birds in Group 1 in addition to the one which died, but none of them were visibly sick.

The results obtained with lactose were less satisfactory than those with dry skim-milk, but still demonstrated that the birds to which it

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was fed were given considerable protection against coccidiosis. As recorded in the table, it was found that the mash consumption in this pen was less than in either of the other two. This is probably a factor responsible for the difference in the effectiveness against coccidiosis afforded by the dry skim milk and lactose mash mixtures.

Another factor that is probably in part responsible for the greater effectiveness of dry skim milk against coccidiosis is the superior food value of this milk product as indicated by the increased growth made by the chicks in Group 1.

This increase in weight amounted to 38.6 grams a chick, or 26.2 per cent more than was made by those in the control pen, which were fed the plain mash, in spite of the fact that the latter consumed 0.4 grams more mash per chick daily than those receiving the dry skim milk mash. The chicks which were fed the lactose mash consumed 12.8 per cent less than those receiving dry skim milk mash and, therefore, attained the least growth.

#### SUMMARY AND CONCLUSIONS

The results of the series of five experiments were uniform in demonstrating that chicks were afforded a considerable degree of protection against coccidial infection when a sufficient amount of lactose or dry skim milk was added to their diet. In the trials carried out under laboratory conditions, this was accomplished equally well by the individual administration of two 1-gram doses of lactose to each bird daily at an interval of about eight hours or by feeding chicks continuously with mash containing 20 per cent lactose or 40 per cent dry. skim milk. In the trials carried out under field conditions, however, the results obtained from the use of skim-milk powder were superior to those obtained from the use of lactose. This was due, at least in part, to the fact that the chicks did not relish the mash mixture containing lactose and, therefore, consumed less of this mash than of that containing dry skim milk. The relatively greater increase in weight of the chicks fed on dry skim milk indicated that the superior food value of this material was also at least in part responsible for the benefit derived from its use.

The results of these experiments, confirm those described in the preceding paper in showing that when sufficient lactose or dry skim milk is fed to chickens, the hydrogen ion concentration of the cecal contents can be kept within a range of 4.4 to 5.6. It is thought that this degree of acidity may be sufficient to injure or destroy the sporo-

zoite or merozoite forms of *Eimeria avium* and that serious harm from the infection is thereby prevented. However, both merozoites and occysts were found in the droppings of birds inoculated with sporulated oöcysts and treated with lactose or dry skim milk even though the birds showed no visible signs of sickness after the inoculation. This is evidence that at least a part of the sporozoites released from the sporocysts were unharmed and invaded the cells of the cecal mucosa where both the sexual and asexual cycles of development were completed. A possible explanation of this is that the dose of sporulated oöcysts given was too large to be entirely overcome and, therefore a portion of the sporozoites escaped. Another possible explanation is that the acidity in the ceca was more destructive to the merozoites than to the sporozoites. In such a case, the invasion of the epithelial cells by the sporozoites and the completion of the developmental cycles within the cells would be unhindered. The merozoites, however, upon emergence from the epithelial cells into the acid cecal contents would be destroyed and further development of disease arrested. On this basis, the appearance of blood in the droppings and death on the fifth and sixth days after inoculation of some of the birds which were fed lactose or skim-milk powder could be ascribed to the tissue damage resulting from the initial invasion with sporozoites. The destruction of the merozoites, however, prevented further development of disease in the birds which were not fatally injured by the sporozoite invasion.

This explanation would not apply to the failure of lactose feeding in the last two coccidiosis control trials, to afford the chicks as high a degree of protection against coccidial infection as was given by dry skim milk. This, as previously pointed out, was probably due in part to the difference in amount of consumption of the two mash mixtures by the chicks (12.8 per cent less of lactose) and also in part to the superior food value of the skim-milk powder.

The fact that feeding chickens mash containing 40 per cent dry skim milk not only protected them against coccidial infection, but also stimulated rapid growth indicates that this would be a valuable practice in the prevention and control of outbeaks of the disease on poultry farms.