A STUDY OF THE CONTROL OF THE DISSEMINATION OF SALMONELLA PULLORUM IN FORCED DRAFT INCUBATORS

by

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INTRODUCTION

The problem, of which the following thesis is a report was divided into two phases, A and B. Phase A consisted of a study of hatchability when using varying degrees of temperature and a constant wet bulb reading of 95°F. during the time of hatch. Phase B was a study of the control of the transmission of Salmonella pullorum organisms from infected to non-infected chicks in a forced draft incubator by high humidity.

The first work undertaken on the problem of the dissemination of S. pullorum in a forced draft incubator was by Hinshaw and his co-workers (1) in 1926. They found that the S. pullorum organism was disseminated by artificially infected chick-down placed in a forced draft incubator. In 1927, Hinshaw, Scott and Payne (2) showed that S. pullorum was transmitted to normal chicks from infected chicks hatched from eggs from reactor hens in a forced draft incubator in six out of eight hatches.

Coon and others (3) in 1928 found that formaldehyde fumigation, using a temperature of 99 to 100°F. and a wet bulb reading of approximately 90°F. would satisfactorily sterilize incubators of the forced air draft type without injuring the hatchability of the eggs. However, because

of the injurious effects upon chicks, fumigating with formaldehyde gas could not be used as a means of control of the disease while hatched chicks were in the machine. They observed in this series of experiments that relative humidity had a very decided influence on the circulation of chick-down. The maintaining of a wet bulb reading of 89 to 90°F. in a Smith incubator resulted in a decided decrease in the number of circulating particles of material as compared to a wet bulb reading of 83°F.

The next step in the control of the dissemination of the disease in the incubator was undertaken by King and others (4) in 1929. They used high humidity as a means of control. From these experiments it was found that a wet bulb reading of 95°F. (relative humidity 84.5 per cent) in the incubator at hatching time practically eliminated the spread of S. pullorum organisms from infected chicks to non-infected chicks hatching in a forced draft incubator. A mortality of 1.2 per cent due to S. pullorum was found in chicks hatched from eggs from non-reacting hens, in the same end of the incubator as chicks hatched from eggs from reacting hens and 0.4 per cent in chicks hatched from eggs from non-reacting hens in the opposite end of the incubator. One disadvantage encountered by the use of high humidity during the hatching period, however, was that hatchability

of the eggs was impaired. They report an average hatchability of 49.8 per cent when a wet bulb reading of 95°F. (relative humidity 84.5 per cent) was used, compared with 58.3 per cent when a wet bulb reading of 85°F. (relative humidity 56.5 per cent) was used. Since it was shown that the dissemination of S. pullorum could be controlled to a very high degree by the use of high humidity during the hatching period, the next desired step was to secure better hatchability while using high humidity at hatching time. Phase A of the following thesis problem, was carried out to determine the effect on hatchability of varying degrees of temperature during the hatching period while using a wet bulb reading of 95°F.

OBJECT OF EXPERIMENT

Phase A

The object of this phase of the experiment was to determine the effect on hatchability of varying degrees of temperature during the hatching period in a forced draft incubator using a wet bulb reading of 95°F.

PROCEDURE

Eggs were incubated weekly from November 21, 1929 to February 28, 1930. Complete hatchability records of each

hatch were taken. The eggs were weighed at four different times during incubation to determine the per cent loss in weight. The temperature of the incubators, room temperatures and wet bulb readings were recorded three times daily. To test livability, the chicks of each hatch were brooded for two weeks. Weight and mortality records were kept for each hatch.

Outline of Experiment

Two Number 9 Buckeye forced draft incubators were used for the experiment. The one was used for the control hatches and operated at the recommended temperature of 100°F and at a wet bulb reading of 85°F. (relative humidity 56.5 per cent) which were considered normal for maximum hatches. The other, or experimental incubator, was operated for the first 20 days at the same degrees of temperature and humidity as the controls. When the first pipped egg was observed, the humidity and temperature were changed according to the following table:

Table I. Outline of Experiment (Phase A) Giving Temperatures and Humidity Readings Used for Each Hatch in Each of the Incubators.

Hatch Number	Experimental: First Nineteen Days of Incubation		Experimental: Twenty and Twenty-First Days of Incubation		Controls: Entire Incubation Period	
	Wet Bulb Reading	Temperature	Wet Bulb Reading	Temperature	Wet Bulb Reading	Temperature
1	(1) 85°F.	100°F.	(2) 95°F.	96°F.	(1) 85°F.	100°F.
2	85°F.	100°F.	95°F.	96°F.	85°F.	100°F.
3	85°F.	100°F.	95°F.	96°F.	85°F.	100°F.
4	85°F.	100°F.	(3) 95°F.	98°F.	85°F.	100°F.
5	85°F.	100°F.	95°F.	98°F.	85°F.	100°F.
6	85°F.	100°F.	95°F.	98°F.	85°F.	100°F.
7	85°F.	100°F.	(4) 95°F.	100°F.	85°F.	100°F.
8	85°F.	100°F.	95°F.	100°F.	85°F.	100°F.
9	85°F.	100°F.	95°F.	100°F.	85°F.	100°F.

(1) Relative Humidity = 56.5 per cent
(2) Relative Humidity = 99.5 per cent
(3) Relative Humidity = 91.5 per cent
(4) Relative Humidity = 84.5 per cent

Source of Eggs

The eggs for this experiment were obtained from a pen of 125 Single Comb Rhode Island Red hens at the college poultry farm. They were mated to 10 cockerels. All the birds of this pen had been carefully tested for several generations and found to be free from S. pullorum infection as determined by the agglutination test. On October 25, 1929 all birds in the pen both males and females, were bled and tested by the tube agglutination method. One bird, hen #1501 gave a positive test. She was removed from the flock.

Setting of Eggs

The hatches were set each Thursday evening at 9 P.M. The eggs set were never more than seven days old. Only eggs of good shell texture and of reasonably good size were used. They were divided equally into the two lots, experimental and control. Caution was taken to have the older eggs equally distributed in the two groups. Just before placing in the incubator, the eggs were weighed as a group and the weight recorded.

Incubator Management

Both incubators were operated and managed as nearly alike as was possible. The experimental incubator was

located in the research laboratory on the second floor of the agricultural building. The control incubator was located in another room across the hall. The temperature and wet bulb reading of both incubators and room temperatures were recorded three times daily. Part of the time a bi-record recording thermometer was used on the experimental incubator to record temperature of incubator and room. (Plate I). All eggs were turned three times daily, morning, noon and night until the close of the eighteenth day. Eggs were weighed when set, and again on the sixth, twelfth and eighteenth days. A seventh day test was made for infertiles and dead embryos. On the eighteenth day, they were again candled for dead embryos and the remaining eggs transferred to the hatching trays. The chicks were taken from the incubator immediately upon completion of the hatch and all dead in shell, pips, cripples and vigorous chicks recorded. The number of sticky chicks and chicks with imperfectly healed navels was recorded for the last six hatches.

Fumigation of Incubators

Both incubators were fumigated at the beginning of the experiment and again between each hatch with 40 grams of potassium permanganate and 80 cubic centimeters of 37 per cent formalin. The fumigating was done immediately after the eggs were placed in the hatching trays to avoid pullorum

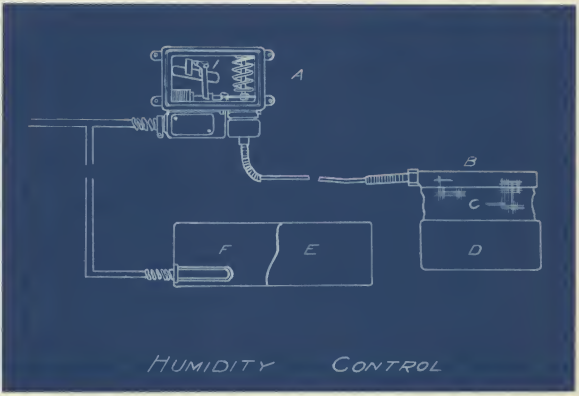
The incubator and equipment used for the experimental hatches.

organisms entering the incubator through the hatching trays. Thus, everything in the incubator had been exposed to the formaldehyde gas at time of hatching. The potassium permanganate crystals were put in a porcelain bowl and the bowl set in the moisture pan in the center of the incubator. The formalin was then poured over the crystals and all doors of the incubator were closed. The fumigating was administered the last thing at night and the incubator not opened again until the following morning. Before fumigating, the humidity was increased to a wet bulb reading of 90°F. and the temperature held normal or at 100°F. The port holes in the incubator were not closed during fumigation.

Moisture Control

In the carrying out of this experiment, it was not only necessary to maintain a definite humidity in both of the incubators but also to be able to regulate it to any degree desired. This was accomplished by the use of a Mercoid Control installed on each of the incubators. The apparatus consisted of a Mercoid Control Switch (A), Figure I, operated by the expansion and contraction of a gas in a tube (B) covered with a wick (C). The lower the humidity in the incubator, the greater the evaporation from the wick. This cooled the gas and caused it to contract which turned on the switch connected to an immersion heater (F) placed in the

Figure I



moisture pan of the incubator. As the temperature of the water increased, more moisture was evaporated from the surface thus increasing the humidity of the incubator. The increased humidity would decrease the amount of evaporation from the wick (C), consequently the gas in the tube surrounded by the wick would expand and break the circuit. This apparatus was very dependable and would hold the wet bulb reading within one degree of the desired point.

A tested Fahrenheit thermometer was used to determine the amount of moisture in the air of the incubator. The bulb of the thermometer was covered with a close fitting wick. The wicks which were renewed each week, were immersed in a small bottle of distilled water.

At the beginning of the first hatch in the experimental incubator, difficulty was encountered in reducing the temperature to 98°F. because of the heat given off by the moisture pan. By placing six additional moisture pans in the incubator and at the same time reducing the amount of moisture given off by the thermoid control system, it was possible to lower the temperature to about 98°F. The temperature was further reduced by adding moisture, drop at a time on the blades of the fan from a large bottle on top of the incubator, (Plate I). From these two sources, sufficient moisture was thus obtained without an excessive amount of heat. The thermoid control system was adjusted accordingly and

used to keep the humidity constant at a wet bulb reading of 95°F. It was also necessary to lower the room temperature approximately ten degrees to get the temperature of the incubator down to the desired point.

It was not necessary to use the bottle of water on top of the incubator or to lower the room temperature for the hatches taken off at 98°F. When the incubator was operated at a temperature of 100°F. during the hatching period, the thermoid control was used as a source of all moisture. The temperature and humidity could be changed to any desired degree and held constant, inside of a period of two hours.

Brooding of Chicks

All chicks from both incubators were brooded for a period of two weeks under as nearly identical conditions as were possible. Upon removal from the incubator, they were immediately placed in small wire bottom brooders for a period of 24 hours. The purpose of placing them in these brooders was to obtain the "chick down" which they would lose during the first 24 hours. The down was collected on clean papers placed underneath each brooder. The object in collecting the chick down was to determine approximately the amount which had been given off by the chicks in each of the incubators. At the end of this 24 hour period, they were weighed and then transferred to an electric brooder on a

table six feet square. The table was divided into four compartments, equal in size. The control groups were too marked to serve as a means of identification in case any of the groups were accidently mixed. The chicks were watered when placed in the brooder and fed the following all-mash chick ration:

Ground yellow corn	45	pounds
Ground wheat	15	"
Ground oat groats	15	"
Meat and bone scraps	14	"
Dried buttermilk	5	"
Alfalfa leaf meal	5	"
Salt	1	"

All chicks were weighed at the age of one day, one week and two weeks. A daily record of mortality was made.

OBSERVATIONS AND RESULTS

A summary of hatchability results, obtained in the experimental and control incubators at the different degrees of relative humidity, is given in the following table:

Table II. Summary of Hatchability Results

	Temp.	Wet bulb	Per cent rel. hum.	Eggs set	Per cent fertile	Per cent hatch fert. eggs
Group I.						
Experimental	96°F.	95°F.	99.5	287	85.4	58.8
Controls	100°F.	85°F.	56.5	287	84.3	59.5
Group II.						
Experimental	98°F.	95°F.	91.5	264	86.0	76.2
Controls	100°F.	85°F.	56.5	264	84.8	76.3
Group III.						
Experimental	100°F.	96°F.	84.5	270	84.8	79.9
Controls	100°F.	85°F.	56.5	270	85.6	71.0

In group I, the 58.8 per cent hatch of fertile eggs in the experimental machine operated at a dry bulb temperature of 96°F. and a wet bulb temperature of 95°F. was slightly less than that obtained from the control incubator (59.5 per cent) operated at optimum conditions. (Table II). The difference however, is too small to be significant. Hatches from both incubators were below normal being due in part, to the season of the year in which the hatching eggs were produced. The eggs used in these hatches were laid in December and January when low hatchability could be expected.

When a dry bulb temperature of 98°F. and a wet bulb temperature of 95°F. (Group II.) were maintained in the experimental incubator during the hatching period, the hatchability of eggs was equal to that obtained in the control incubator. The per cent hatch of fertile eggs in the former was 76.2 while in the latter it was 76.5.

In the last group of three hatches taken off at a dry bulb temperature of 100°F. and a wet bulb reading of 95°F. the per cent hatch was 79.9 while in the controls it was 71.0. A larger percentage of chicks were obtained using this higher temperature, but as will be seen later, the quality of these chicks was not as good as it was in the chicks from the control hatches. King (8) using a temperature of 100°F. during the entire incubation period obtained the highest per cent hatchability (58.3) when eggs were hatched at a wet bulb reading of 85°F. At a wet bulb reading of 75°F. the next best hatchability (56.4) was obtained. The poorest hatchability (48.8) was obtained when the wet bulb reading was 95°F.

The time of first chick in each of the nine hatches is given in Table XII. The period shown in days and hours represents the time elapsing between the setting of the eggs and the first hatched chick.

Table III. Time of Hatch

	Period in Days and Hours				
	Hatch 1	Hatch 2	Hatch 3	Average	Difference
Group I..					
Experimental	20 da- 0 hr	20 da- 8 hr	20 da-22 hr	20 da-10 hr	
Control	19 da-20 hr	19 da-14 hr	19 da-18 hr	19 da-17 hr	17 hr
	Hatch 4	Hatch 5	Hatch 6		
Group II.					
Experimental	20 da-16 hr	20 da- 7 hr	20 da- 5 hr	20 da- 9 hr	
Control	19 da-18 hr	19 da-21 hr	19 da-23 hr	19 da-21 hr	12 hr
	Hatch 7	Hatch 8	Hatch 9		
Group III.					
Experimental	20 da-10 hr	20 da- 2 hr	19 da-20 hr	20 da- 3 hr	
Control	20 da- 1 hr	19 da-22 hr	20 da- 0 hr	20 da- 0 hr	3 hr

When the temperature was lowered to 96°F. during the hatching period, the hatches on the average were 17 hours later than the control hatches, (Table III). On account of this retarding of the hatch, it does not seem practical to use a temperature of 96°F. during the hatching period. The first chick was observed in the experimental group of Hatch I at about the same time as in the control. This hatch had been exposed to the low temperature during only one period. Hatch 2, however, was still later having been exposed to the low temperature twice. Hatch 3 was considerably later because of the fact it had been exposed to the 96°F. temperature for three different periods.

The group of hatches taken off at a temperature of 98°F. were on the average 12 hours later than the controls. Part of this difference can be accounted for because Hatch 4 had been exposed twice to the 96°F. temperature of the first three hatches and Hatch 5 was exposed to it once. If all three hatches had been exposed to the 96°F. temperature for the three hatching periods, probably the difference in time of hatch would not have been as much.

The experimental hatches of the last group, hatched at a temperature of 100°F., were three hours later than the controls. Here again, Hatches 7 and 8 had been exposed to a lower temperature and as a result were later in hatching than Hatch 9 or the controls.

The number and per cent of "sticky"* chicks and chicks with imperfectly healed navels occurring in the last six hatches of the experiment are given in the following table:

Table IV. The Number and Per Cent of Abnormal Chicks

	Group No. 1 (Three hatches)				Group No.II (Three hatches)				Group No.III (Three hatches)			
	Exp.		Con-trols		Exp.		Con-trols		Exp.		Con-trols	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
"Sticky" chicks	-	-	-	-	2	1.2	4	2.3	24	13.1	1	0.6
Incompletely healed navels	-	-	-	-	14	8.1	11	6.4	43	23.5	13	7.9

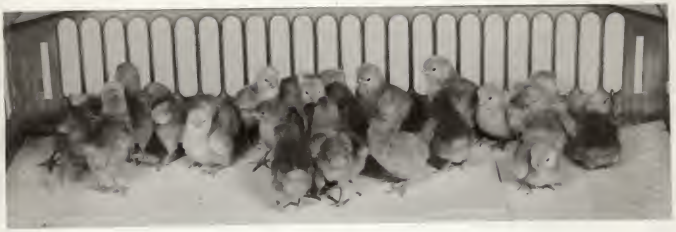
To be consistent throughout the experiment, chicks showing signs of stickiness or of imperfectly healed navels but strong and vigorous were included in the number of chicks hatched. The number of "sticky" chicks and chicks with imperfectly healed navels occurring in each of the last six hatches were recorded in a separate table (Table IV). No record was taken for the first three hatches as no noticeable difference existed in the quality of the chicks from the two incubators.

* The term "sticky" chicks refers to a chick the down of which has not fluffed out. The down adheres to the body in clumps. Sometimes pieces of shell or shell membrane are stuck fast to the chick's back. They appear to be normal in every other respect.

Townsley (5) states that "cleaner hatches" are produced with fewer "sticky" chicks when using a relatively high humidity in the incubator, providing the temperature is correct. In accordance with his work, it was observed in this experiment, (Table IV, Group II), that when the temperature was lowered two degrees while using a high humidity, the hatches were as good in quality as were the control hatches. However, when the temperature was maintained at 100°F. with high humidity, 12.5 per cent more "sticky" chicks and 15.6 per cent more chicks with imperfectly healed navels occurred in the experimental group of hatches than in the controls.

To illustrate that no difference existed in the quality of chicks hatched in the two incubators, photos were taken of 25 representative chicks from each of the groups at the age of one day, one week, and two weeks, (Plates II and III). The experimental chicks were hatched in a relative humidity of 91.5 per cent and the controls in a relative humidity of 56.5 per cent.

The average weights in grams of the chicks at the age of one day, one week and two weeks are given in the following table:

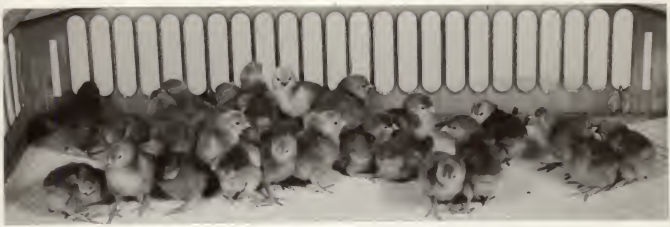


One Day Old

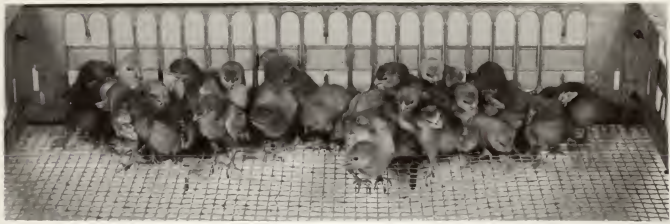
One Week Old

Two Weeks Old

Twenty-five of the experimental chicks hatched in a relative humidity of 91.5 per cent.



One Day Old



One Week Old

Two Weeks Old

Twenty-five of the control chicks hatched in a relative humidity of 56.5 per cent.

Table V. Average Weight Per Chick in Grams

Group	No. of hatch	One Day Old		One Week Old		Two Week Old	
		Exp.	Con-trols	Exp.	Con-trols	Exp.	Con-trols
I	1	32.6	32.6	42.5	42.2	62.9	65.8
	2	34.9	31.8	41.1	39.7	59.0	58.7
	3	37.1	34.0	41.4	40.5	63.5	61.1
	Average	34.9	32.8	41.7	40.8	61.8	61.9
II	4	36.6	37.4	43.4	45.9	69.5	68.6
	5	39.1	38.6	45.4	44.2	67.2	66.6
	6	39.7	37.4	45.9	43.7	69.2	65.8
	Average	38.4	37.8	44.9	44.6	68.6	66.5
III	7	39.4	38.6	42.5	41.4	66.9	64.9
	8	38.0	36.6	45.9	44.2	69.2	65.2
	9	36.6	37.4	43.7	41.4	64.4	63.2
	Average	38.7	37.5	44.0	42.3	66.8	64.4

In all of the hatches, except two, the chicks from the high humidity incubator were heavier than those from the control incubator, (Table V). In one of the two exceptions the chicks averaged the same in weight while in the other, the control chicks were heavier. Taking the average of each group of three hatches, the chicks hatched in the high humidity were heavier chicks at one week of age than those hatched in the lower humidity. At two weeks of age, the experimental chicks of Groups II and III still averaged better in weight than did the control chicks. The chicks of Group I from both incubators avereged approximately the same at the age of two weeks.

The weight of the eggs set did not influence the weight of the chicks hatched for in Group I the eggs set in both the experimental and control incubators averaged 56.8 grams. In Group II the average weight in the experimental hatches was 57.0 grams while in the control, it was 57.4 grams. The control eggs were again slightly heavier than the experimental eggs in the last group of hatches. In the former, the average weight was 57.7 grams while in the latter it was 57.5 grams.

The per cent mortality occurring in the experimental and control chicks during the first two weeks of life are given in Table VI.

Table VI. Per Cent Mortality

Group	No. of hatch	First Week Exp.	First Week Controls	Second Week Exp.	Second Week Controls	Total Exp.	Total Controls
I	1	5.0	2.5	2.5	2.5	7.5	5.0
	2	7.0	0.0	0.0	4.7	7.0	4.7
	3	2.9	8.0	2.9	4.0	5.8	12.0
II	4	2.0	0.0	0.0	0.0	2.0	0.0
	5	4.0	0.0	0.0	2.0	4.0	2.0
	6	0.0	0.0	2.0	0.0	2.0	0.0
III	7	0.0	6.8	4.5	0.0	4.5	6.8
	8	2.0	0.0	2.0	0.0	4.0	0.0
	9	4.0	4.0	0.0	0.0	4.0	4.0
Total per cent mortality for Group No. I						6.8	7.2
Total per cent mortality for Group No.II						2.6	0.7
Total per cent mortality for Group No.III						4.2	3.6
Average per cent mortality for 3 groups						4.5	4.9

A total of 34 chicks, or 4.1 per cent of the 839 chicks brooded, died during the experiment. No excessive mortality was encountered in either of the two lots in any of the hatches, (Table VI). The difference in per cent mortality of the chicks from the experimental and control incubators was not large enough to be of any significance. The chicks from both incubators were about equal in livability.

Loss in Weight of Eggs During Incubation

The eggs in the control machine of this experiment incubated at a relative humidity of 56.5 per cent, lost 9.4 per cent of their total weight during incubation. This was the average loss in weight for the nine hatches. The weight of the eggs used, averaged 57.3 grams. Lamson and Kirkpatrick (6) using results obtained from incubating some 10,000 eggs found a 9.8 per cent loss in weight of eggs incubated at a relative humidity of between 50 and 60 per cent. The average initial weight of the eggs they used was 57 grams. Townsley (5) obtained a per cent evaporation loss of 9.9 when using a relative humidity of 56 per cent.

The average loss in weight and per cent hatch of fertile eggs for the experimental hatches were as follows:

Group Number	Per cent Rel. Hum.	Per cent Loss In Weight	Per cent Hatch Fert. Eggs
1	99.5	5.0	58.8
2	91.5	7.8	76.2
3	84.5	8.4	79.9

The relative humidity during the incubation period when eggs were not hatching, was the same as in the controls or 56.5 per cent. The maximum hatchability was obtained when the loss in weight was 8.4 per cent. Lamson and Kirkpatrick (6) obtained maximum hatchability (69.3 per cent of fertile eggs) when the loss in weight of eggs set was 9.8 per cent. When loss in weight was reduced to 8.7 per cent, they found that the hatches were slightly reduced. The per cent hatch of fertile eggs obtained was 62.1. With a per cent loss in weight of 5.3, the per cent of fertile eggs hatched was 45.5. They used small still air machines and carried out the experiment under entirely different conditions from those under which this experiment was conducted.

In a series of experiments on incubation, Eycleshymer (7) found that the evaporation could be lessened until the eggs lost but 9 per cent of their original weight and still give healthy chicks. He did not report per cent hatchability in his results. He came to the conclusion that the incubator should be so controlled that it would allow the evaporation of about 13 per cent of the original weight of the egg. Eycleshymer (7) as well as Lamson and Kirkpatrick

(6) were using the same relative humidity during the entire incubation period.

A Study of Chick Down

King (8) observed that after chicks hatch in an incubator, there was a large number of flaky particles floating in the air and deposited on the floor of the machine. This was incorrectly spoken of as "chick down". In reality only a small part of it was made up of down from the chick. A close study of a chick just hatched revealed the down in small clumps. He found each clump to be surrounded by a capsule or shield. As the chick dries and comes in contact with other objects, it breaks the capsule and allows the clump of barbs to separate. This procedure is commonly known as fluffing out. The "chick down" found in the incubator after a hatch was over was discovered to be made up, to a very large extent, of these broken capsules.

When a high humidity was provided in the incubator while the chicks were hatching, King (8) observed that the fluffing out process was retarded. To check on the extent to which chicks fluff out in the incubator at different degrees of relative humidity, the down from approximately 150 chicks hatched at the three different degrees of relative humidity was collected as described under procedure. It was found that as the relative humidity was increased,

the amount of "down" given off by the chicks in the incubator was decreased. (Plate IV).

The amount of "down" given off by chicks in the incubator is important because it was shown by Hinshaw and others (1) that S. pullorum organisms were transmitted to non-infected chicks from artificially infected chick down placed in a forced draft incubator. When an egg infected with S. pullorum hatches, the chick is surrounded by a suspension of the organisms. The capsules breaking off from the down undoubtedly contain many germs. These circulate through the air and infect, usually through the respiratory organs, non-infected chicks hatching in the same incubator.

DISCUSSION

From the foregoing observations and results obtained from the incubation of 1,642 eggs and the brooding of 839 chicks (279 hatched), it seems that to secure satisfactory hatchability of livable chicks while using a wet bulb reading of 95°F. at hatching time, the temperature should be lowered from 100 to 98°F. in a Buckeye Number 9 forced draft incubator. Townsley (5) working with a Smith forced draft incubator normally operated at a temperature of 99°F., comes to the conclusion that as the humidity is increased, the temperature requirement is reduced. The wet bulb readings he used were 75, 85 and 90°F. Three hatches were incubated

Plate IV

Group I Group II Group III

The amount of "chick down" collected during the first 24 hours of brooding from 150 chicks hatched at different wet bulb readings.

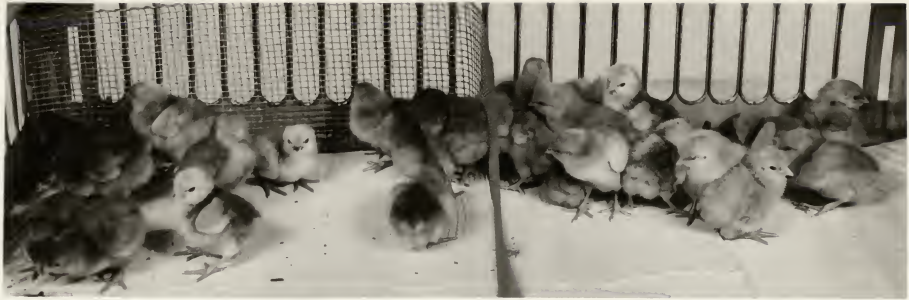
at the three degrees of humidity using a dry bulb temperature of 99°F. in each case. After the third hatch, the incubator with high humidity was operated at 98°F. and the one with low humidity raised to 100°F. In the medium moisture machine, the temperature was left at 99°F. He states that the adjustment of temperature evened up the time required to complete the hatch in each of the three machines. It also produced noteworthy changes in the per cent of hatch, in size of chicks, and livability of the chicks from the incubator at high relative humidity. He further adds that bad results commonly blamed on too much humidity are caused by too high a temperature, and that there is little danger of getting too much humidity if the temperature is properly adjusted. It seems that the proper balance between humidity and temperature is very important.

A survey of the literature indicates a lack of accurate data on the question of what degrees of humidity and temperature to use during incubation to secure the most satisfactory hatchability. A number of workers have made comparative tests on the hatching results obtained in incubators, with and without moisture added, but very few have hatched at varying degrees of relative humidity. Atwood, Dryden, Brooks, Lewis and others have pointed out that the addition of moisture to the air of the incubator generally improves the hatches. It has also been shown by Graham

that chicks hatched in machines with moisture added, lived better than those hatched in incubators to which moisture had not been added. It seems that humidity does play a very important part in successful incubation. In view of the present magnitude of the hatchery industry, it seems surprising that more data are not available.

An interesting observation made during the experiment was the difference in appearance in the chicks from the high humidity incubator and those from the control incubator. The control chicks had a silky appearance while the chicks hatched in the high humidity were coarse or rough in the down when first taken from the incubator. However, they would lose the coarse appearance after being in the brooder a short time. This coarse appearance was probably due to the down retaining part of the capsules as explained under the discussion of chick down. Another marked difference between the two lots of chicks was that the experimental chicks were slightly lighter in color than were the controls when taken from the incubator, (Plate V). They were also lighter in color at two weeks of age.

Plate V



Controls

Experimental

Relative Humidity 56.5 per cent

Relative Humidity 91.5 per cent

HATCHABILITY SUMMARY OF THE INDIVIDUAL HATCHES

Group No. I

	Wet Bulb Reading 95°F. Dry Bulb Reading 96°F.					
	Hatch No.1		Hatch No.2		Hatch No.3	
	Experi-mental	Con-trols	Experi-mental	Con-trols	Experi-mental	Con-trols
No. Eggs Set	96	96	96	96	95	95
No. Eggs Broken During Hatch	0	3	0	3	2	2
% Eggs Broken During Hatch	0	3.8	0	3.7	2.5	2.3
No. Infertiles	17	16	16	15	9	14
% Infertiles	17.7	16.7	16.7	15.6	9.5	14.7
Dead Germ (1)	5	3	7	12	6	4
% Dead Germ (1)	6.3	3.8	8.8	14.8	7.0	4.9
Dead Germ (2)	6	10	3	8	27	7
% Dead Germ (2)	7.6	12.5	3.8	9.9	31.4	8.6
Dead in Shell	7	9	11	10	12	11
% Dead in Shell	8.9	11.3	13.8	12.3	14.0	13.6
No. Pips	6	5	2	5	4	5
% Pips	7.6	6.3	2.5	6.2	4.7	6.2
No. Cripples	1	0	2	0	0	1
% Cripples	1.3	0	2.5	0	0	1.2
No. Fertiles	79	80	80	81	86	81
% Fertility	82.3	83.3	83.3	84.3	90.5	85.3
No. Chicks	54	50	55	45	35	51
% Hatch of Fertile Eggs	68.4	62.5	68.8	55.1	40.7	63.0

Group No. 2

	Wet Bulb Reading 95°F. Dry Bulb Reading 98°F.					
	Hatch No.4		Hatch No.5		Hatch No.6	
	Experimental	Controls	Experimental	Controls	Experimental	Controls
No. Eggs Set	87	87	93	93	84	84
No. Eggs Broken During Hatch	0	0	0	0	0	1
% Eggs Broken During Hatch	0	0	0	0	0	1.4
No. Infertiles	15	15	17	13	7	12
% Infertiles	14.9	17.2	18.3	14.0	8.3	14.3
Dead Germ (1)	7	6	5	7	3	5
% Dead Germ (1)	9.5	8.3	6.6	8.8	3.9	6.9
Dead Germ (2)	4	3	1	3	0	1
% Dead Germ (2)	6.4	4.2	1.3	3.8	0	1.4
Dead in Shell	4	3	3	6	9	4
% Dead in Shell	5.4	4.2	3.9	7.5	11.7	5.6
No. Pips	3	4	4	2	5	3
% Pips	4.1	5.6	5.3	2.5	6.5	4.2
No. Cripples	2	2	4	2	0	1
% Cripples	2.7	2.8	5.3	2.5	0	1.4
No. Fertiles	74	72	76	80	77	72
% Fertility	85.1	82.8	81.7	86.0	91.7	85.7
No. Chicks	54	54	59	60	60	57
% Hatch of Fertile Eggs	73.0	75.0	77.6	75.0	77.9	79.2

Group No. 3

	Wet Bulb Reading 95°g. Dry Bulb Reading 100°F.					
	Hatch No.7		Hatch No.8		Hatch No.9	
	Experi- mental	Con- trols	Experi- mental	Con- trols	Experi- mental	Con- trols
No. Eggs Set	84	84	93	93	93	93
No. Eggs Broken During Hatch	0	0	0	0	0	0
% Eggs Broken During Hatch	0	0	0	0	0	0
No. Infertiles	12	16	15	14	14	9
% Infertiles	14.3	19.0	16.1	15.1	15.1	9.7
Dead Germ (1)	2	6	1	3	4	7
% Dead Germ (1)	2.8	8.3	1.3	3.8	5.1	8.3
Dead Germ (2)	2	3	1	0	1	2
% Dead Germ (2)	2.8	4.4	1.3	0	1.3	2.4
Dead in Shell	8	3	2	4	6	5
% Dead in Shell	11.1	4.4	2.6	5.1	7.6	6.0
No. Pips	8	8	5	11	2	4
% Pips	11.1	11.8	6.4	13.9	2.5	4.8
No. Cripples	0	2	1	5	3	4
% Cripples	0	2.9	1.3	6.3	3.8	4.8
No. Fertiles	72	68	78	79	79	84
% Fertility	85.7	81.0	83.9	84.9	84.9	90.3
No. Chicks	52	46	68	56	63	62
% Hatch of Fertile Eggs	72.2	67.7	87.2	70.9	79.7	73.8

OBJECT OF EXPERIMENT

Phase B

The object of the second phase of the experiment was to determine the amount of dissemination of Salmonella pullorum from infected chicks to non-infected chicks in a forced draft incubator in which the humidity during the hatching period corresponded to a wet bulb reading of 95°F. and the temperature held at the degree giving the most satisfactory hatchability as determined in Phase A.

PROCEDURE

Since it was found that by lowering the temperature two degrees, satisfactory hatchability could be secured when using high humidity during the time of hatch, the next desired step was to find to what extent the pullorum disease was transmitted from infected chicks to non-infected chicks at hatching time in high humidity. To determine this, four hatches were completed at a temperature of 98°F. and a wet bulb reading of 95°F. (relative humidity 91.5 per cent).

To determine the minimum amount of moisture which could be used at hatching and still control the transmission of the disease, three additional hatches were taken off at a dry bulb temperature of 100°F. and a wet bulb temperature of

90°F. (relative humidity 68.0 per cent).

The eggs were incubated weekly from February 20, 1930 to April 25, 1930. The total number of eggs incubated was 3,100. Complete hatchability record of each hatch was taken. The temperature of the incubators, room temperatures and wet bulb readings were recorded three times daily. The chicks of each hatch were brooded for a period of two weeks. Weight and mortality records were kept. Each hatch was divided into the four following groups:

Group I. Chicks hatched from eggs from reactor hens.

Group II. Chicks hatched from eggs from non-reactor hens in the same end of the incubator as Group I.

Group III. Chicks hatched from eggs from non-reactor hens in the opposite end of the incubator from Group I.

Group IV. (Control Group) Chicks hatched from eggs from non-reactor hens in another incubator operated at optimum conditions of humidity and temperature.

Source of Eggs

The non-infected eggs in Groups II, III, and IV were produced by the flock of Rhode Island Red hens used in

phase A. The infected eggs for Group I were obtained from a flock of Rhode Island Red hens all of which had reacted to the agglutination test. The infected hens were housed approximately one-half mile from the pullorum disease free stock. Both flocks were fed and managed alike.

Incubator Management

The same incubators were used and managed in the same manner as in the first part of the experiment. Both incubators were fumigated between each hatch as outlined in phase A. The hatches were set each Thursday evening at 9 P.M. Approximately two trays of the eggs from infected hens and three trays of the eggs from the non-infected hens were set each week. A seventh day test was made for infertiles and dead embryos. On the eighteenth day, they were again candled for dead embryos, and the remaining eggs transferred to the hatching trays. The chicks were taken from the incubator immediately upon completion of the hatch and a record taken of the number of dead in shell, pips, cripples, and vigorous chicks.

Brooding of Chicks

During the experiment, 2,062 chicks were brooded for a period of two weeks. They were weighed when taken from the incubator, watered and then placed in small wire bottom

brooders. Fifty watt electric light bulbs were used in each brooder as a source of heat. Each group was toe-marked for identification. The first feed was placed before them when twenty-four hours old. The same ration used in the first part of the experiment was fed. Feed and water was before them at all times. Every morning, clean papers were placed under each brooder. Group I was brooded in a separate room from the others and every precaution was taken to prevent contamination from one group to the other.

Autopsy of Chicks

An autopsy was held on the 239 chicks which died during the experiment. This was held on the day of their death unless they died late in the evening in which case they were held over in the ice box until the following day. Cultures were taken from the liver, lungs, unabsorbed yolk and bone marrow and grown on nutrient salts agar for 24 hours. All growths resembling that of S. pullorum were isolated and grown in semi-solid sugar media for 24 hours. Chicks yielding organisms that formed either acid or acid and gas in dextrose but which did not break down the lactose, maltose or sucrose were recorded as having died from the pullorum disease.

The agar medium used in culturing the organisms consisted of a mineral mixture containing the following:

Agar	15-20 grams
Ammonium dihydrogen phosphate	0.6 "
Potassium bicarbonate	0.5 "
Potassium citrate	2.0 "
Sodium chloride	2.9 "
Ferric ammonium citrate	2.5 "
Glucose (not more than)	0.5 "
Glycerol	5.0 "
Peptone	20.0 "
Water	1000.0 cc.

The neutrality of the medium was adjusted to a pH value of 7.2 by the use of N/1 sodium hydroxide. Usually not more than 20 cubic centimeters of the N/1 sodium hydroxide were required per liter. The medium was heated in a steamer for one hour and tubed without filtering. The sugar free medium used in identifying the S. pullorum organism consisted of a mineral mixture containing the following:

Peptone	20.0 grams
Potassium bicarbonate	0.5 "
Ammonium dihydrogen phosphate	0.6 "
Disodium dihydrogen phosphate	1.7 "
Ferric ammonium citrate	2.5 "
Agar	1.0 "
Water	1000.0 cc.

About 12 cubic centimeters of a 0.02 per cent aqueous solution of brom-thymol blue were added. The pH was then adjusted with N/1 sodium hydroxide to give the medium a deep grass green color. When this color was obtained, the pH value of the medium was about 7.0. After tubing, the medium was sterilized. Two cubic centimeters of 10 per cent sterile solutions of the four sugars were added to each tube. The

tubes were then incubated for 24 hours and all contaminated tubes discarded. The above media were devised by Scott (13).

OBSERVATIONS AND RESULTS

The following table gives a summary of the hatchability obtained in each of the four groups at two different degrees of humidity:

Table VII. A Summary of the Hatchability Results

Number of Hatches	Temp.	Wet Bulb	Rel. Hum.	Eggs Set	Per cent Fertile	Per cent Hatch Fert. eggs
Group I.						
4	98°F.	95°F.	91.5%	606	88.0	70.2
3	100°F.	90°F.	68.0%	568	88.6	71.2
Group II.						
4	98°F.	95°F.	91.5%	354	91.8	75.5
3	100°F.	90°F.	68.0%	288	88.2	76.4
Group III.						
4	98°F.	95°F.	91.5%	354	93.2	76.4
3	100°F.	90°F.	68.0%	288	90.3	75.2
Group IV.						
4	100°F.	85°F.	56.5%	354	91.5	72.2
3	100°F.	85°F.	56.5%	288	91.7	76.0

Both reactors and non-reactor flocks gave very good fertility of hatching eggs, (Table VII). The hatchability of fertile eggs from the reactor hens was slightly less

than it was from the pullorum disease free stock. There was not the difference in hatchability that was found by other workers. Beaudette and others (9) while working with the pullorum disease found the fertile eggs from infected hens gave a 53 per cent hatch while those from non-infected hens gave a 65 per cent hatch. Probably one of the reasons why a greater difference was not obtained in this experiment was that the eggs from the reactor hens averaged 6.4 grams per egg heavier and were of much better shell texture than those from the non-reactor hens. The difference in eggs produced was very likely in the stock and not due to any difference in the way the birds were fed and managed. Attention should be called to the fact that hatchability in Groups II and III (eggs from non-reactor hens hatched in a relative humidity of 91.5 per cent), was slightly higher than the controls hatched in a relative humidity of 56.5 per cent. They were approximately equal in hatchability when a relative humidity of 68.0 per cent was used in the experimental incubator. These results check very closely with the hatchability results obtained in phase A, (Table II, Group II).

The number and per cent of mortality and mortality due to S. pullorum occurring in the seven hatches at high and low relative humidities are presented in the following table:

Table VIII. A Summary of Mortality

Number of Hatches	Wet Bulb Read.	Number of Chicks	Mortality No.	Mortality Per cent	Mortality due to S.pullorum No.	Mortality due to S.pullorum Per cent
Group I.						
4	95°F.	374	90	24.1	80	21.4
3	90°F.	358	44	12.3	41	11.5
Group II.						
4	95°F.	239	33	13.8	12	5.0
3	90°F.	194	12	6.2	1	0.5
Group III.						
4	95°F.	252	19	7.5	5	2.1
3	90°F.	195	19	9.8	1	0.5
Group IV.						
4	85°F.	235	13	5.5	0	0.0
3	85°F.	206	9	4.4	0	0.0

At a wet bulb reading of 95°F. (relative humidity 91.5 per cent), the pullorum disease was transmitted from the diseased chicks to the healthy chicks to the extent of 5.0 per cent when hatched in the same end of the incubator and 2.1 per cent when hatched in the opposite end of the incubator, (Table VIII). When the relative humidity was lowered to 68 per cent (wet bulb reading 90°F.), 0.5 per cent mortality due to S. pullorum occurred in each of the groups hatched from eggs from non-reactor hens and exposed to infected chicks in the same incubator. One explanation for the decrease in the amount of dissemination of the disease when the relative hu-

midity was decreased is that there was 9.9 per cent more infection and 11.8 per cent higher mortality in the infected hatches taken off at the high humidity than in those taken off at the low humidity. It may be that chicks hatched in high humidity are less able to withstand the ravages of the disease than they are when hatched in a lower humidity. However, before any definite conclusions can be drawn as to the minimum amount of moisture which can be used during time of hatch for satisfactory hatchability and still control the dissemination of the disease, more work should be carried out.

King (8) using a relative humidity of 84 per cent obtained in the group hatched in the same end of the incubator as the infected chicks, a mortality due to S. pullorum of 1.2 per cent as compared with 12.8 in a 55 per cent humidity. In the chicks hatched in the opposite end of the incubator at the high humidity, the per cent mortality due to S. pullorum was 0.4 per cent as compared with 14.3 at the low humidity. In the infected group at relative humidities of 55 and 84, the mortality per cents occurring from S. pullorum infection were 12.5 and 11.4 respectively. Although a slightly greater amount of dissemination occurred in this experiment while using high humidity, the results compare favorably with those of King.

The S. pullorum organism was not isolated from any of the 22 chicks (5.0 per cent) which died in the control group.

The mortality was not excessive in any one hatch indicating further that disease was not present. It was observed throughout the experiment that when mortality above normal occurred in the hatches exposed to the disease, the S. pullorum organism was certain to be found.

The following table gives the per cent distribution of the S. pullorum organism occurring in the four regions of chick's body from which cultures were taken.

Table IX. The Distribution of the Organism

	Per Cent S. pullorum Occurring in Each Region			
	Liver	Lung	Unabsorbed Yolk	Bone Marrow
Infected Chicks	73	57	80	69
Exposed Chicks	79	84	42	75

As stated in the procedure, cultures were taken from the liver, lungs, unabsorbed yolk and bone marrow. In 62 per cent of the chicks upon which an autopsy was held, the S. pullorum organism was isolated from all four regions. Lung lesions were very frequently found in the chicks exposed to the disease. The organism was isolated from the lungs in 84 per cent of the exposed chicks, (Table IX). In many of the chicks exposed to S. pullorum infection, more especially in the older chicks, the yolk was almost completely absorbed, while in the infected chicks, the yolk

was very seldom completely absorbed.

It seems that when a chick becomes infected with the disease through the lungs, the organisms do not reach the yolk as readily as when the chick is naturally infected through the egg. This is shown by the fact that the organism occurred in the unabsorbed yolk in 80 per cent of the infected chicks while in the exposed chicks, it was isolated from 42 per cent.

DISCUSSION

Pullorum disease in chicks is an acute septicemic disease caused by the organism Salmonella pullorum. Eggs laid by hens with the disease may contain S. pullorum and chicks hatched from such eggs may be infected. This is the most common source of infection in chicks. They may also acquire the disease from contaminated incubators. This latter source of infection can be eliminated by fumigation of the incubator with formaldehyde gas as shown by Coon and his coworkers (8) in 1928. Fumigating the incubator is of value only in preventing infection from carrying over from one hatch to the next. It has not been successful in preventing the transmission of the disease from infected to non-infected chicks hatching simultaneously in the same machine. Bunyea and Hall (10) reached the conclusion that the fumigation of incubators during the hatching period does not

overcome the transmission of pullorum disease from infected to non-infected chicks, since 51 per cent of the normal exposed chickens that died, (11.5 per cent of the entire normal exposed hatch) showed the infection.

Since it has been shown that it is by means of the chick down that the disease is disseminated from infected to normal chicks and further that the amount of solid material in the air of an incubator is considerably reduced when moisture is added, high humidity seems to offer a very good means of control. As shown by King (8) and from the results obtained in this experiment, high humidity in the incubator at hatching time will greatly reduce the transmission of the disease. This method is not one hundred per cent effective but it is high enough to be of value in controlling the spread of the disease in commercial hatcheries. The results obtained in this experiment indicate that an incubator temperature of 98°F. and a wet bulb temperature of 95°F. or an incubator temperature of 100°F. and a wet bulb temperature of 90°F., at hatching time, are sufficient to check the dissemination of the S. pullorum organisms in the type of incubator used. The additional moisture above this latter degree appears to be of little value in so far as control of the disease is concerned.

CONCLUSIONS

1. Satisfactory hatchability can be secured from eggs hatched at a temperature of 98°F. and a wet bulb reading of 95°F. The relative humidity at the above degrees is 91.5 per cent.

2. Heavier chicks are produced when hatched in a relative humidity of from 84.5 per cent to 99.5 per cent than at 56.5 per cent humidity.

3. The chicks hatched in high relative humidity were less silky in appearance and lighter in color than the chicks hatched in a low relative humidity.

4. There was no significant difference in the livability of the chicks hatched in varying degrees of humidity.

5. When using a wet bulb reading of 95°F. during the hatching period, fewer "sticky" chicks and chicks with imperfectly healed navels were produced at a temperature of 98°F. than at 100°F.

6. A temperature of 96°F. with a wet bulb reading of 95°F. at hatching time proved to be impractical. Such temperatures retard the hatch 17 hours.

7. As the relative humidity of the incubator is increased, the amount of "chick down" liberated from hatched chicks is decreased.

8. The per cent loss in weight of eggs during incubation varies inversely with the per cent relative humidity.

9. As the humidity is increased during the hatching period, the temperature requirement is decreased.

10. If the temperature is properly adjusted, there is little danger of providing too much humidity in the incubator at time of hatching.

11. An incubation temperature of 100°F. and a wet bulb temperature of 90°F. at hatching time, practically eliminated the dissemination of S. pullorum organisms from infected chicks to normal chicks in the type of incubator used.

12. The dissemination of the disease can also be controlled at a temperature of 98°F. and a wet bulb reading of 95°F.

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APPENDIX

A Summary of the Individual Hatches

	Groups	No. Set	Fertility No.	Fertility Per cent	Fert. Eggs No.	Fert. Eggs Per cent	Mortality No.	Mortality Per cent	Mortality due to S.pullorum No.	Mortality due to S.pullorum Per cent
Hatch 10										
	1	93	78	83.9	52	66.7	3	5.7	0	0.0
	2	69	65	94.2	45	69.2	1	2.2	0	0.0
	3	69	62	89.9	46	74.2	2	4.3	0	0.0
	4	69	65	94.2	45	69.2	2	4.4	0	0.0
Hatch 11										
	1	129	97	75.2	64	66.0	24	37.5	21	32.8
	2	96	85	88.5	57	67.1	4	7.0	0	0.0
	3	96	88	91.7	67	76.1	5	7.5	0	0.0
	4	96	89	92.7	66	74.2	1	1.5	0	0.0
Hatch 12										
	1	192	177	92.2	128	72.3	36	28.1	35	27.3
	2	93	84	90.3	64	76.2	16	25.0	6	9.4
	3	93	91	97.8	74	81.3	8	10.8	5	6.8
	4	93	82	88.2	59	72.0	5	8.5	0	0.0
Hatch 13										
	1	192	181	94.3	130	71.8	27	20.8	24	18.5
	2	96	91	94.8	73	80.2	12	16.4	6	8.2
	3	96	89	92.7	65	73.0	4	6.2	0	0.0
	4	96	88	91.7	64	72.7	5	7.8	0	0.0

A Summary of the Individual Hatches

	Groups	No. Set	Fertility No.	Fertility Per cent	Fert. Eggs No.	Fert. Eggs Per cent	Mortality No.	Mortality Per cent	Mortality due to S.pullorum No.	Mortality due to S.pullorum Per cent
Hatch 14										
	1	192	169	88.0	131	77.5	25	19.0	24	18.3
	2	96	85	88.5	73	85.9	3	4.0	0	0.0
	3	96	83	86.5	68	81.9	3	4.4	0	0.0
	4	96	90	93.8	76	84.4	3	3.9	0	0.0
Hatch 15										
	1	186	164	88.2	112	68.3	7	6.3	7	6.3
	2	96	83	86.5	65	78.3	7	10.8	1	1.5
	3	96	86	89.6	65	75.6	5	7.7	0	0.0
	4	96	89	92.7	67	75.3	2	3.0	0	0.0
Hatch 16										
	1	190	170	89.5	115	67.6	12	10.4	10	8.7
	2	96	86	89.6	56	65.1	2	3.6	0	0.0
	3	96	91	94.8	62	68.1	11	17.7	1	1.6
	4	96	85	88.5	63	66.6	4	6.3	0	0.0