

THE PREVENTION OF THE DISSEMINATION OF
SALMONELLA PULLORUM IN FORCED
DRAFT INCUBATORS

by

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INTRODUCTION

Bacillary white diarrhea is a very destructive disease of chicks caused by the organism Salmonella pullorum. It is transferred from the hen to the egg and thus to the chick upon hatching. The greatest mortality is caused in chicks between four and fourteen days of age.

Hinehaw, Upp and Moore (1926) showed chicks could become infected with S. pullorum in the incubator by breathing artificially inoculated chick down. Scott (1927) proved the disease was also transferred from infected chicks to non-infected chicks in the incubator at hatching time. Gwathin (1926) and Coon (1926) used formaldehyde gas to prevent this dissemination of infection. Fumigation required a high moisture content to make the gas effective. Periodic fumigation was advantageous, but an experiment was planned to determine the effect of high humidity alone on the dissemination of S. pullorum in forced draft incubators.

OBJECT OF EXPERIMENT

The object of this experiment was to determine the effect of varying degrees of humidity in forced draft incubators at hatching time, upon the dissemination of

S. pullorum from infected chicks to non-infected chicks.

PROCEDURE

A. Source of Eggs

The eggs used in this experiment were obtained from two flocks of Single Comb Rhode Island Reds, fed and managed alike. One flock contained 80 birds all of which reacted to the agglutination test. This flock was housed separate from all other birds. The other flock consisted of 95 non-reacting hens on the college poultry farm which had been carefully tested for several generations and found to be free from S. pullorum infection as determined by the agglutination test. None of the chicks from this flock died of Bacillary white diarrhea, during the experiment unless exposed to infected chicks in the incubator.

B. Classification of Groups

In each hatch, at least one tray of eggs from the reactor hens, and one tray of eggs from the non-reacting hens were hatched in one end of a forced draft type incubator. In the opposite end of the incubator another tray of eggs from the non-reacting flock was hatched. The controls were eggs from the non-reacting flock,

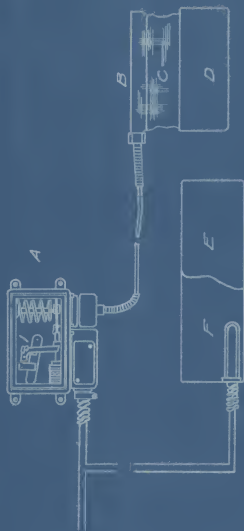
incubated in the same machine until the 18th day of incubation when they were transferred to a separate incubator in another room.

For simplicity the following classification will be used in discussing the experiment.

- Group 1. Chicks hatched from eggs from reactor hens.
- Group 2. Chicks hatched from eggs from non-reacting hens in the same end of the incubator as group 1.
- Group 3. Chicks hatched from eggs from non-reacting hens in the opposite end of the incubator from group 1.
- Group 4. Control chicks hatched from eggs from non-reacting hens in another incubator.

C. Moisture Control

In studying this problem it was necessary to maintain a definite humidity in the incubator and to be able to regulate it to any degree desired. The apparatus used consisted of a mercoid switch (1) operated by the expansion and contraction of a gas in a tube (B) covered with a wick (C) and saturated with water from pan (D). 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 a water bath



HUMIDITY CONTROL

(E) in the incubator. As the water neared the boiling point the amount of moisture evaporating from the surface of the water increased and the humidity in the incubator increased causing less evaporation from the wick. The gas would then get warm and expand, turning the switch off.

This apparatus was very dependable and would hold the wet bulb reading within two degrees of the desired point.

A tested Fahrenheit thermometer with the bulb covered by a clean wick and saturated with distilled water was used as an indicator of the moisture content of the incubator.

D. Disinfecting

The incubator was disinfected in the beginning and between each hatch with 40 grams of potassium permanganate and 80 cubic centimeters of 40 per cent formalin. The potassium permanganate was placed in a porcelain basin over the water pan in the center of the incubator. The formalin was then poured over the crystals. The heat generated from the chemical action was sufficient to boil the formalin causing formaldehyde gas to be liberated. In all cases the humidity was maintained above a wet bulb reading of 85°F. While disinfecting, the incubator was

allowed to run in the normal manner.

E. Technique of Examining Eggs

The eggs were placed in a five per cent phenol solution for fifteen minutes, then washed with 95 per cent alcohol and any alcohol remaining on the egg was ignited. A further sterilization of the shell was secured by flaming the large end of the egg until it turned black. With a pair of sterile forceps a hole about 1/2 centimeter in diameter was made in the shell. A culture was taken from the yolk of the egg and streaked out on a meat infusion agar plate with a sterile loop. The plates were incubated for twenty-four hours and the growth, if characteristic of *E. pullorum*, was identified by growing on the four sugars; glucose, lactose, maltose, and sucrose.

F. Hatching of Eggs

Twelve different hatches were taken off. The first four were hatched at a wet bulb reading of 95°F. (relative humidity 84 per cent). Due to the small amount of dissemination of infection, the next four hatches were taken off at a wet bulb reading of 75°F. (relative humidity 32 per cent), to see if the humidity was the controlling factor. As this seemed to be the case, two more

hatches were taken off at a wet bulb reading of 95°F. (relative humidity 84 per cent) to further check the effect of high humidity. The last two settings were hatched at a wet bulb reading of 85°F. (relative humidity 55 per cent), to determine the amount of dissemination occurring at this humidity. The hatching period extended from February 15, 1929 to June 8, 1929. (See appendix for a summary of the individual hatches).

Eggs were set Friday evening of each week and turned each morning and evening until the 18th day of incubation when they were candled and all infertile eggs and those containing dead embryos were removed. The remaining eggs were moved to the hatching trays, and the control eggs were transferred to another incubator. During all of the hatches the temperature was held at 100°F. The chicks were taken from the incubator twenty-four hours after the peak of the hatch.

G. Brooding the Chicks

The chicks were all brooded for two weeks in Liv-an-Gro brooders using electric light bulbs as a source of heat. Group 1 was brooded in a different room from the other groups. The trays underneath the brooders, containing droppings, were cleaned each morning, and every means of precaution was used to prevent contamination

from one group to the other.

H. Rations and Management

The chicks received the revised Kansas all-mash chick feed, consisting of:

Yellow corn	45 pounds
Wheat	15
Oat groats	15
Meatscraps	14
Dried buttermilk	5
Alfalfa leaf meal	5
Cod liver oil	<u>1</u>
	100

They received their first feed when forty-eight hours old and from then on mash and fresh water were continually before them.

I. Autopsy of Chicks

The 397 chicks that died during the experiment were autopsied on the day of their death, with exception of a few times, when they were held over in an ice box. Cultures from the liver, unabsorbed yolk, and sometimes from lung lesions, were taken and grown on plain nutrient agar for twenty-four hours. All growths resembling that of S. pullorum were isolated and grown on one per cent semisolid sugar media for twenty-four hours. Chicks yielding organisms that formed acid and gas on glucose and did not react with lactose, maltose, or sucrose were

recorded as having died from Bacillary white diarrhea.

OBSERVATION

A. Study of Bacterial Flora of Incubator

Coon (1927) found the number of organisms in the air of an incubator was greater when the wet bulb reading was 75°F. than when it was 85°F. or 95°F. His work was done with an empty forced draft incubator. Unpublished work by Townsley (1929) when chicks were hatching, seemed to disagree with Coon's work.

For the purpose of checking the work done by Coon, plates were exposed for varying lengths of time in an incubator at different periods while chicks were hatching. The results obtained varied somewhat, but showed a smaller number of organisms in the air at a wet bulb reading of 95°F. than at 75°F. A summary that coincides with Coon's results is shown in Table I.

Table I. Summary of Bacterial Flora of an Incubator at various wet bulb readings.

Wet bulb reading	Number of colonies on plates exposed			total
	one min.	five min.	fifteen min.	
80°	133	287	541	951
85°	67	226	401	714
95°	64	145	278	487

B. Study of Chick Down

It has been observed that after chicks hatch in an incubator there are a large number of flaky particles floating in the air and deposited on the floor of the machine. This is often incorrectly spoken of as "chick down". In reality only a small part of it is made up of down from the chick. A close study of a chick just hatched will reveal the chick down in small clumps. Each clump is surrounded by a capsule or shield. As the chick dries, and brushes itself against other objects, it breaks this capsule and allows the clump of barbs to separate. The procedure is known as fluffing out. The broken capsule in the form of irregular pieces then makes up a large part of the "chick down" found in the incubator after the hatch is over. (See Plate II).

When an egg infected with S. pullorum hatches the chick is surrounded by a suspension of the organisms. Each flake of this capsule then contains many germs. These fly thru the air and infect normal chicks hatching in the same incubator.

If a high humidity is provided in the incubator while the chicks are hatching the fluffing out process is retarded. When the chicks are removed from the incubator

Plate II. Chick Down (x 12)



11



A525B

Plate III. Chick down collected from chicks hatched at the three wet bulb readings.

11a



twenty-four hours after the peak of the hatch, the bulk of the "chick down" will be deposited in the brooder where conditions are less crowded and not so conducive to the spread of the disease. This is illustrated by Plate III showing the amount of "chick down" collected during the first 44 hours under a wire bottom brooder from 50 chicks hatched under various wet bulb readings. The chicks were removed from the incubator twenty-four hours after the peak of the hatch, and placed undisturbed in Liv-an-Gro brooders.

C. Bacteriology of Eggs

In order to determine what per cent of the eggs laid by the reactor hens, carried S. pullorum, 243 eggs from the first six hatches were examined. Table II summarizes the results.

Table II. Summary of Bacteriological Examination of Eggs from Reactor Flock.

Hatch	Infertiles examined	Infected No.	%	Dead Germs examined	Infected No.	%
1	33	1	3.03	50	0	0.00
2	13	1	7.69	22	6	27.27
3	11	1	9.09	13	3	23.07
4	24	2	8.33	12	1	8.33
5	18	3	16.66	12	5	41.66
6	<u>25</u>	<u>2</u>	<u>8.00</u>	<u>10</u>	<u>2</u>	<u>20.00</u>
	124	10	8.07	119	17	14.30

Total eggs examined 243
 Total number of infected eggs 27
 Total per cent infected eggs 11.11

The infertile eggs revealed 8.07 per cent carried S. pullorum. The dead germ eggs examined showed 14.30 per cent contained S. pullorum. Of the total eggs tested 11.11 per cent were found to be infected.

Scott (1927) examined 871 eggs and found 10.44 per cent were infected. Runnells and Van Roekel (1927) reported that of 306 eggs laid by reacting Single Comb White Leghorns and examined for S. pullorum 14 per cent were infected. Of 169 eggs produced by Rhode Island Reds infection was found in 33 per cent. Kaup and Dearstynne (1927) reported from examinations made of 3,818 eggs laid by reactor hens 5.94 per cent of the eggs carried S. pullorum.

D. Hatchability

The hatchability results of the different groups are presented in Table III.

The per cent hatchability was best when the eggs were hatched at a wet bulb reading of 85°F. The next best hatches were obtained when the wet bulb reading was 75°F. at hatching time, and the poorest hatchability was obtained when the wet bulb reading was 95°F.

The average per cent hatch in group 4 was low because of one or two real poor hatches due to incubator management, and probably to the moving of the eggs on the 18th

day from one machine to another.

Table III. Summary of Hatchability Results.

	Wet bulb or Hum.	Rel. Hum. %	No. eggs	Tested out		Hatchability	
				No.	%	No.	%
Group 1.	75°F.	32 %	716	117	16.3	410	57.2
	85°F.	55 %	285	43	16.9	168	59.4
	95°F.	84 %	644	141	21.8	271	42.1
Group 2.	75°F.	32 %	354	50	14.1	202	57.0
	85°F.	55 %	192	46	23.9	117	60.9
	95°F.	84 %	465	103	22.1	249	53.5
Group 3.	75°F.	32 %	354	62	17.5	195	55.0
	85°F.	55 %	192	47	24.4	105	54.7
	95°F.	84 %	489	128	26.2	249	50.8
Group 4.	--	--	354	58	16.3	167	47.2
	--	--	192	55	28.6	105	54.7
	--	--	489	123	25.1	192	39.2

It may not be practical for all commercial hatcheries to maintain a wet bulb reading of 95°F. at the time the hatch is coming off for several reasons. First, in the Smith Mammoth Incubators where hatches are taken off twice each week, the eggs would be exposed to such a high humidity five times during the hatch, that it would perhaps be difficult to dry them down sufficiently to hatch good. Second, when the humidity was maintained at such a high point for a two day period the incubator doors on the side of the machine used in this experiment swelled and made it almost impossible to get into the incubator. Continued high moisture caused the sides of the incubator to warp and, unless the field coils in the motor are properly

protected they will corrode.

K. Mortality Results

The mortality results of this experiment are summarized and presented in Table IV.

Table IV. Summary of Mortality Results.

Net bulb	No. chicks	Mortality		Mortality due to B. W. D.	
		No.	%	No.	%
Group 1.					
75°	410	123	30.00	91	22.19
85°	168	25	14.88	21	12.50
95°	271	47	17.34	31	11.43
Group 2.					
75°	202	47	23.26	40	19.80
85°	117	15	12.82	15	12.82
95°	249	14	5.62	3	1.20
Group 3.					
75°	195	68	34.87	48	24.61
85°	105	18	17.14	15	14.28
95°	248	17	6.85	1	.40
Group 4.					
--	464	23	4.95	0	0.00

During the experiment none of the 464 chicks from group 4 died from Bacillary white diarrhea, and only 4.95 per cent died from other causes.

It will be noted that the mortality in group 1 did not decrease as the moisture of the incubator was increased at hatching time. The reason for this is that

a large per cent of the mortality in this group was due to infection received direct from the egg. The mortality then would depend upon the number of infected eggs that hatched.

The highest mortality was 34.87 per cent, occurring in group 3 hatched at a wet bulb reading of 75°F. This was reduced to 17.14 per cent for chicks hatched at a wet bulb reading of 85°F. and 6.85 per cent when the wet bulb reading was maintained at 95°F. while the chicks were hatching.

The same results were obtained in group 2 where the mortality was 23.26 per cent when the chicks were hatched at a wet bulb reading of 75°F., 12.82 per cent at a wet bulb reading of 85°F. and only 5.62 per cent when the wet bulb was maintained at 95°F. when the chicks were hatching.

This shows very clearly that as the moisture is increased the total mortality is decreased and the deaths due to Bacillary white diarrhea are lessened.

The probable error of the difference is shown in Table V.

The probable error of the mean was calculated by the following formula in which F equals success, f equals failure, and N equals number.

$$P. E. M. \quad .6745 \sqrt{\frac{F \times f}{N}}$$

The probable error of the difference was determined from:

$$\sqrt{(E_1)^2 + (E_2)^2}$$

in which E_1 represents the first error, and E_2 stands for the second error.

Table V. The Probable Error of the Difference on the Mortality Results.

% Mort. at 75°F.	Difference between 75°F. - 85°F.	% Mort. at 85°F.	Difference between 85°F. - 95°F.	% Mort. at 95°F.
Group 1. 30.00	15.12 ± 2.3	14.83	-2.46 ± 2.4	17.34
Group 2. 23.26	10.44 ± 2.8	12.82	7.20 ± 3.7	5.62
Group 3. 34.97	17.73 ± 3.3	17.14	10.29 ± 4.1	6.85

DISCUSSIONS AND CONCLUSIONS

Up to the present time, there is only one recognized way of controlling Bacillary white diarrhea, and that is by means of the agglutination test. By using this test and removing the birds that show a positive reaction, one can eliminate the infected birds from the breeding pens. Repeated testing together with proper sanitary measures will eventually eliminate the disease from the flock. The cost and inconvenience of this test makes it impracticable for everyone to have their breeding birds tested, this is especially true of many of the small

flocks that supply hatching eggs to the commercial hatcheries. For this reason the hatcheries that use eggs from many sources for the production of baby chicks and for custom hatching are the principle source of infection. They distribute, not only the chicks that receive the infection from the egg, but those that have been exposed to the disease in the incubator. In view of these facts the dissemination of infection in the incubator has become an important factor in the control of the disease.

Coon (1927-28) used formaldehyde gas as a means of preventing this dissemination of infection. Fumigation, when used according to the methods outlined, will disinfect the incubator very thoroughly and can be used between hatches to prevent the infection from carrying over from one hatch to the next very successfully. The reason for not using it while the chicks are hatching to prevent dissemination of the disease is because of the severity of this gas on the chicks. Exposure of the chicks to the gas for more than a couple of hours proved fatal.

Now that high moisture in the incubator at hatching time has been found to practically eliminate the spread of the disease from infected chicks to non-infected chicks, the commercial hatcheries can use this new method without fear of injury to the chicks. The only disadvantage of using a wet bulb reading of 95°F. while the chicks are

hatching is that it lowers the per cent hatchability. During this experiment 829 eggs hatched at 95°F., gave 57 per cent hatchability of total eggs set. The 1,597 eggs hatched at a wet bulb reading of 95°F., gave 48 per cent hatchability. This shows a difference of 9 per cent \pm 2.77. The difference is perhaps significant, but a large amount of work is yet to be done along the line of high humidity. By lowering the temperature one degree Townsend (1929) succeeded in getting better hatches and larger chicks when the wet bulb reading was 90°F., than at any other humidity. Lomson and Kirkpatrick (1918) in very extensive work showed the per cent hatch of fertile eggs did not decrease until the relative humidity in the incubator throughout the entire hatch was above 60 per cent. This would compare to a wet bulb reading of 87°F. To the authors knowledge, so far no work has been carried out to test the effect of various degrees of humidity, at hatching time, upon the per cent hatchability. Until work along this line has been checked one will have to expect a small decrease in hatchability when a 95°F. wet bulb reading is used. This loss is not great, however, and hatcheries that are having complaints from Bacillary white diarrhea chicks would no doubt be willing to stand the small loss in number of chicks if they could be sure the dissemination of Salmonella pullorum was prevented.

SUMMARY OF RESULTS

1. A wet bulb reading of 95° F. in the incubator at hatching time practically eliminated the spread of Bacillary white diarrhea from infected chicks to non-infected chicks hatching in a forced draft incubator.

2. Mortality in chicks hatched from reactor eggs to two weeks of age at the three different wet bulb readings were:

75° F. - 30.00 per cent
 85° F. - 14.88 per cent
 95° F. - 17.34 per cent

3. Mortality in chicks from eggs laid by non-reacting hens, hatched in the same end of the incubator as eggs from reactor hens, at the three different wet bulb readings were:

75° F. - 23.26 per cent
 85° F. - 12.82 per cent
 95° F. - 5.62 per cent

4. Mortality in chicks from eggs laid by non-reacting hens, hatched in the opposite end of the incubator from eggs from reactor hens, at the three different wet bulb readings were:

75° F. - 34.87 per cent
 85° F. - 17.14 per cent
 95° F. - 6.85 per cent

5. Mortality in the control chicks, hatched in another incubator from eggs laid by non-reacting hens, was 4.95 per cent.

6. S. pullorum was not isolated from any of the control chicks that died.

7. S. pullorum was isolated from 8.07 per cent of the infertile eggs laid by the reactor hens.

8. S. pullorum was isolated from 14.30 per cent of the reactor eggs that died in the shell.

9. S. pullorum was isolated from 11.11 per cent of the 243 eggs examined.

10. There are less organisms in the air of an incubator when the wet bulb reading is at 95°F. than when it is at 75°F.

11. There is less "chick down" liberated in the incubator from chicks hatching at a wet bulb reading of 95°F. than at 75°F.

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APPENDIX

A Summary of the Individual Hatches

Wet Bulb	Group	No. Set	Infertiles and Dead Embryos	Chicks Hatched	Mortality No.	Mortality %	Mortality due to B. W. D. No.	Mortality %
Hatch number one:								
95° F.	1	96	33	15	15.5	16.3	0	0.0
	2	75	17	36	48.0	8.3	0	0.0
	3	75	22	29	38.6	6.8	0	0.0
	4*	75	21	15	17.5	7.6	0	0.0
Hatch number two:								
95° F.	1	40	13	6	15.0	16.6	1	15.0
	2	48	12	32	66.6	6.2	0	0.0
	3	48	13	25	52.1	4.0	0	0.0
	4*	48	13	25	52.1	6.0	0	0.0
Hatch number three:								
95° F.	1	93	11	40	43.0	0.0	0	0.0
	2	73	11	40	54.8	7.5	0	0.0
	3	96	16	54	56.2	7.4	0	0.0
	4*	96	24	17	17.7	5.8	0	0.0
Hatch number four:								
95° F.	1	96	24	34	35.4	2.9	0	0.0
	2	77	13	47	61.0	2.1	0	0.0
	3	77	14	46	59.7	10.8	0	0.0
	4*	78	13	33	42.3	0.0	0	0.0
Hatch number five:								
75° F.	1	95	20	43	45.2	34.0	9	16.9
	2	95	10	50	52.6	20.0	10	20.0
	3	95	18	45	47.5	40.0	10	22.2

Wet Bulb	Group	No. Set	Infertiles and Dead Embryos		Chicks Hatched		Mortality		Mortality due to B. W. D.	
			No.	%	No.	%	No.	%	No.	%
Hatch number five:										
	4*	95	20	21.0	6	6.3	1	16.6	0	0.0
Hatch number six:										
75° F.	1	227	40	17.6	127	55.9	24	18.9	19	14.9
	2	96	16	16.6	59	61.4	5	8.4	4	6.7
	3	96	16	16.6	49	51.0	9	18.3	3	6.1
	4*	96	9	9.3	67	69.8	2	2.9	0	0.0
Hatch number seven:										
75° F.	1	203	27	13.3	156	67.0	50	36.0	40	29.4
	2	83	9	10.8	52	62.6	15	28.0	12	23.0
	3	83	14	16.8	56	67.4	24	42.8	20	35.7
	4*	83	15	18.0	48	57.8	8	16.6	0	0.0
Hatch number eight:										
75° F.	1	191	30	15.7	104	54.5	34	32.6	23	22.1
	2	80	15	18.7	41	51.2	17	41.4	14	34.1
	3	80	14	17.5	45	56.2	17	37.7	15	33.5
	4*	80	14	17.5	46	57.5	1	2.1	0	0.0
Hatch number nine:										
95° F.	1	173	32	18.4	92	53.1	26	28.2	18	19.5
	2	96	21	21.8	50	52.0	2	4.0	1	2.0
	3	96	27	28.1	42	43.7	3	7.1	1	2.3
	4*	96	29	30.0	47	48.9	0	0.0	0	0.0
Hatch number ten:										
95° F.	1	146	28	19.1	86	48.9	17	19.7	12	13.9
	2	96	29	30.0	44	45.8	3	6.8	2	4.5
	3	96	26	27.0	52	54.1	2	3.8	0	0.0
	4*	96	23	23.9	57	59.3	1	1.7	0	0.0
Hatch number eleven:										
85° F.	1	164	25	15.2	98	49.7	18	18.3	16	16.3
	2	96	20	20.0	62	64.5	11	17.7	11	17.7

Wet Bulb	Group	No. Set	Infertiles and		Chicks		Mortality		Mortality due	
			Dead No.	Embryos %	Hatched No.	Hatched %	No.	%	No.	%
Hatch number eleven:										
	3	96	22	22.9	51	53.1	8	15.6	7	13.7
	4*	96	29	30.0	57	59.3	0	0.0	0	0.0
Hatch number twelve:										
85° F.	1	119	23	19.3	70	48.9	7	10.0	5	7.1
	2	96	26	27.0	55	57.2	4	7.2	4	7.2
	3	96	25	26.0	54	56.2	10	18.5	8	14.8
	4*	96	26	27.0	48	50.0	4	6.9	0	0.0

A Summary of the Total Hatches at:

95° F.	1	644	141	21.8	271	42.1	47	17.3	31	11.4
	2	465	103	22.1	249	53.5	14	5.6	3	1.2
	3	488	128	26.2	249	50.8	17	6.8	1	.4
	4*	489	123	25.1	192	39.2	7	3.7	0	0.0
85° F.	1	283	48	16.9	168	59.4	25	14.8	21	12.5
	2	192	46	23.9	117	60.9	15	12.8	15	12.8
	3	192	47	24.4	105	54.7	18	17.1	15	14.2
	4*	192	55	28.6	105	54.7	4	3.8	0	0.0
75° F.	1	716	117	16.3	410	57.2	123	30.0	91	22.2
	2	354	50	14.1	202	57.0	47	23.2	40	19.8
	3	354	62	17.5	195	55.0	68	34.8	48	24.6
	4*	354	58	16.3	167	47.2	12	7.1	0	0.0
Total		4,723	978	20.7	2,429	51.4	397	16.3	265	10.9

* Group 4 was hatched in each case as near optimum humidity as possible.