A STATISTICAL EVALUATION OF SEVERAL CODING SYSTEMS USED TO DETERMINE THE DEGREE OF INFESTATION OF THE SHAFT LOUSE, *MENOPON GALLINAE* (LINN.) ON CHICKENS

BY

JOHN C. HARSHBARGER III

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TABLE OF CONTENTS

INTRODUCTION AND OBJECTIVES

Much research in recent years has been directed toward materials and methods of controlling lice which infest chickens. In order to measure the effectiveness of various materials and methods from the standpoint of control, it is necessary to be able to accurately estimate the degree of louse infestation. To accomplish this, numerous coding systems have been devised and used. A coding system in this case is a method whereby numerical symbols are used arbitrarily to represent different louse infestations as they appear on chickens.

These coding systems have not been previously analyzed statistically to determine their accuracy. Therefore, the necessary linear relationship between the numerical symbols and the infestations of lice they arbitrarily represent can only be assumed.

The primary objective of this study was to analyze statistically several coding systems in current use to determine if there is a linear relationship between the numerical symbols and the degrees of louse infestation they arbitrarily represent. The procedure used to collect the data for this correlation was to score, by several coding systems, the degree of louse infestation on a number of chickens with different degrees of infestation. The total population of lice on these chickens was then removed and counted.

Several techniques have been used to remove and count total populations of parasites from birds but none are completely applicable to this problem. Therefore, a second objective was to develop a technique, more efficient than any published to date, for removing and counting the total louse population.

The shaft louse, Menopon gallinae (Linn.), was used to this work because of convenience, because it is one of the most common poultry parasites, and because this information will be immediately applicable to work being done at the Virginia Agriculture Experiment Station, Blacksburg, Virginia.

REVIEW OF LITERATURE

Coding Systems:

The need for some type of coding system or scale for research workers to use in determining the degree of infestation of lice on individual birds is very evident when one realizes that an individual bird may harbor a population of lice numbering several thousands. Thompson and Hosking (1957), after examining under a steromicroscope every feather except the primary wing feathers of a heavily infested chicken, reported a population of 8270 lice. The need for such a coding system is even more evident when one realizes that many research workers in attempting to estimate total populations of lice on heavily infested birds arrived at figures very much lower. For example, Creighton, et al. (1943) and Creighton, et al. (1947) defined heavily infested birds as those harboring populations of lice ranging from 75-100 individuals. Fairchild and Dahn (1955) estimated entire populations of lice per bird and had estimates that ranged from 32-400 individuals. Raun (1956) attempted to count all the lice on one side of individual chickens. His counts ranged from 23-288 lice. Moore (1952) and Moore and Schwardt (1954) also tried to estimate the number of lice on one side of a bird and to within a two-inch radius of the vent. They reported that a heavy population was one that contained more than one

HUNDRED INDIVIDUALS. WARREN, ET AL. (1948) REPORTED THAT A HEAVY INFESTATION MEANS 300-1000 LICE PER BIRD.

PARMAN, ET AL. (1928) STATED, "UNFORTUNATELY THERE IS NO WAY OF DETERMINING WITH ACCURACY THE NUMBER OF LICE ON A FOWL, BUT FAIRLY CONSISTENT RESULTS ARE OBTAINABLE IF AN EXPERIENCED OBSERVER ESTIMATES THE NUMBER AFTER HAVING CAREFULLY EXAMINED ALL PARTS OF EACH FOWL." EDGAR, ET AL. (1949) STATED SIMILARLY, "IT IS REALIZED, OF COURSE, THAT ANY METHOD OF ATTEMPTING TO COUNT LIVING LICE IS SUBJECT TO CONSIDERABLE ERROR AND CAN BE LITTLE MORE THAN AN ESTIMATE. HOWEVER, WHEN A ROUTINE METHOD OF EXAMINATION IS USED AND THE SAME OPERATOR INSPECTS ALL BIRDS, THE DEGREE OF ACCURACY IS SUCH THAT COMPARISONS BETWEEN PENS CAN BE MADE."

MANY CODING SYSTEMS HAVE BEEN USED BY RESEARCH WORKERS. EDGAR, ET AL. (1949) AND EDGAR AND KING (1950) USED AN EIGHT POINT SYSTEM AS FOLLOWS: (0) NO VISIBLE LICE, (1) VERY LIGHT INFESTATION, (2) LIGHT INFESTATION, (3) MEDIUM LIGHT INFESTATION, (4) MEDIUM INFESTATION, (5) MEDIUM HEAVY INFESTATION, (6) HEAVY INFESTATION, AND (7) VERY HEAVY INFESTATION. EDGAR AND KING STATED THAT THEY OBSERVED EACH CHICKEN FOR APPROXIMATELY ONE MINUTE. PARMAN, ET AL. (1928) USED A SEVEN POINT SYSTEM AS FOLLOWS: NO LICE, VERY FEW, FEW, LIGHT, MEDIUM, HEAVY, AND VERY HEAVY. ALICATA, ET AL. (1946) AND ALICATA, ET AL. (1947) USED A SIX POINT SYSTEM AS FOLLOWS: (0) NO VISIBLE LICE, (1) 1-10 LICE, (2) 10-100 LICE, (3) 1-2 LICE PER SQUARE INCH, (4) 3-5 LICE PER SQUARE INCH, AND

(5) 6 or more lice per square inch. Moore and Schwardt (1954), Reid, et al. (1956), and Linkfield and Reid (1958) all appeared to have used the same six point system as follows: (0) no visible lice, (1) 1-10 slight, (2) 10-25 light, (3) 25-50 medium, (4) 50-100 abundant, and (5) over 100 heavy. Creighton, et al. (1943) used a five point system as follows: (0) no visible lice, (1) light 1-25, (2) medium 25-50, (3) heavy 50-75, and (4) very heavy 75-100 or more. Several experimenters have used four point systems. Telford (1944) used the following system: (0) no visible lice, (light) up to approximately 100, (medium) proportionally more, and (heavy) proportionally more. Hamilton (1942), Warren (1945), and Telford (1947) used a four point system defined only as: none, light, medium, and heavy infestations. Raffensperger (1958a, 1958b) used the following four point system: (0) no visible lice, (1) very difficult to find, 1-2 seen per bird, (2) medium infestation, and (3) heavy infestation.

Techniques for making complete counts:

Several techniques which have been used by research workers in the past to determine the total population of lice on an individual chicken, are outlined in the literature.

Dunn (1932) developed a method of collecting a total population of lice from live birds. A bird with its wings

TIED BEHIND ITS BACK WAS PLACED IN A LARGE JAR. A TOWEL WAS TIED AROUND ITS NECK AND LET DRAPE DOWN AS A BARBERS TOWEL WOULD BE ARRANGED. CHLOROFORM WAS POURED ON THE TOWEL AND THE VAPOR, BEING HEAVIER THAN AIR, SANK TO THE BOTTOM STUPEFYING THE LICE PRESENT. THE BIRD WAS REMOVED FROM THE JAR, HELD OVER A SHEET OF WHITE CLEAN PAPER AND VIGOROUSLY BRUSHED, WHICH EASILY REMOVED THE LICE.

FLOYD AND TOWER (1956) DEVELOPED A METHOD WHICH THEY CONSIDERED SUCCESSFUL. A METAL HATCHING TRAY WAS PLACED OVER A PIECE OF SMOOTH WRAPPING PAPER. A BIRD WHICH HAD ITS WINGS LOCKED OVER ITS BACK WAS PLACED ON THE TRAY AND AN ASSISTANT FIRMLY HELD THE BIRD'S LEGS. APPROXIMATELY THREE TABLESPOONFULS OF PYRETHRUM DUST WAS RUBBED INTO THE FEATHERS. THIS IRRITATED THE LICE PRESENT, CAUSING THEM TO MOVE TO THE OUTSIDE OF THE FEATHERS WHERE THEY COULD BE KNOCKED OFF BY LIGHTLY BRUSHING THE FEATHERS WITH THE HAND. THE BRUSHING PROCESS WAS CONTINUED AS LONG AS THE LICE CONTINUED TO MIGRATE TO THE OUTSIDE OF THE FEATHERS OR APPROXIMATELY TWENTY-FIVE MINUTES. AFTER THE LICE DROPPED FROM THE BIRD TO THE PAPER, THE CONTENTS OF THE PAPER (LICE, FEATHER DEBRIS, PYRETHRUM DUST, ETC.) WAS PLACED IN A VIAL AND LABELED FOR FUTURE COUNTING. NO STATEMENT WAS MADE IN THE ARTICLE EXPLAINING HOW IT WAS KNOWN THAT ALL LICE HAD BEEN REMOVED WHEN THEY COULD NO LONGER BE SEEN MIGRATING TO THE SURFACE OF THE FEATHERS.

When the counts were to be made, each vial was screened (meshes 10-40) to separate the lice from most of the debris. The lice were then transferred to a 200 cc. beaker containing a 100 cc. mixture of water and a small amount of some wetting agent. The mixture was shaken well and poured rapidly through a suction filter which left the lice and remaining debris scattered uniformly over the filter paper. The filter paper was transferred to the turntable of a slide mount ringer. Over the turntable was fixed a glass plate to which was cemented black radiomat lantern slide binding tape. In this black area, five perfect circles exactly 1 cm. in diameter, so arranged as to give a view of any spot on the paper from the center to the outer edge, were cut. The center circle was spaced exactly over the center of the filter paper and the outer circle extended to the outer edge. The slide mount ringer was placed under a stereomicroscope and the turntable was spun with the forefinger. The lice in the center circle were counted first, followed by each distal circle in sequence until all of the lice on the filter paper had been counted.

Hopkins (1949) developed a procedure for removing total populations of ectoparasites from small rodents by soaking the skin for 15 minutes in a 5% solution of KOH, scraping the hair off, and then dissolving the hair completely, leaving only the lice. Cook (1954) modified Hopkins' method

TO PRODUCE GREATER ACCURACY; BEER AND COOK (1957) MODIFIED IT STILL FURTHER TO MAKE IT USEFUL ON BIRDS. AN INFESTED BIRD WAS KILLED AND PLACED IN A POLYETHYLENE BAG. A SMALL QUANTITY OF ETHER WAS ADDED TO KILL THE LICE. AFTER THE LICE WERE DEAD, THE BIRD WAS SKINNED ON A PIECE OF CLEAN WHITE PAPER AND THE SKIN WAS PLACED IN A 1% SOLUTION OF TRYPSIN BUFFERED TO A PH OF ABOUT 8.3 WITH Na_2HPO_4. THIS WAS PLACED IN AN OVEN AND HEATED FOR 12-24 HOURS AT A TEMPERATURE OF 38°C. WHEN THE SKIN WAS REMOVED FROM THE OVEN, KOH WAS ADDED TO DISSOLVE THE FEATHERS AND REST OF THE SKIN. THE MIXTURE WAS BROUGHT SLOWLY TO A BOIL, COOLED AND BROUGHT TO A BOIL AGAIN. AFTER COOLING, THE MIXTURE WAS POURED THROUGH A 200 MESH BRONZE SCREEN FOLDED IN A CONICAL FORM. THE RESIDUE ON THE SCREEN WAS WASHED WITH DISTILLED WATER TO REMOVE ANY REMAINING SOAP OR PIGMENT. THE SCREEN WAS INVERTED INTO A PETRI DISH AND THE ECTOPARASITES WERE WASHED OFF BY A STREAM OF WATER FROM A WASHING BOTTLE. THE SCREEN WAS EXAMINED UNDER A STEREOMICROSCOPE FOR ANY PARASITES THAT MAY HAVE BEEN STILL REMAINING. THE PARASITES IN THE PETRI DISH WERE COUNTED WITH THE AID OF A STEREOMICROSCOPE.

SEVERAL OTHER TECHNIQUES, BOTH FOR COLLECTING AND FOR COUNTING, WHICH WERE NOT FOUND IN THE LITERATURE WERE TRIED IN THE COURSE OF GETTING THE MOST APPLICABLE TECHNIQUE FOR THE PROBLEM AT HAND.

STATISTICAL TECHNIQUE:

OSTLE (1956) OUTLINED THE PROCEDURE FOR SEVERAL OF THE STATISTICAL TESTS USED FOR THIS WORK. THESE INCLUDED ANALYSIS OF VARIANCE, BARTLETT'S TEST FOR HOMOGENITY OF VARIANCES, AND CORRELATIONS. DUNCAN (1955) OUTLINED A PROCEDURE HE DEVELOPED FOR TESTING DIFFERENCES BETWEEN SEVERAL MEANS IN AN ANALYSIS OF VARIANCE. KRAMER (1956) EXTENDED DUNCAN'S METHOD TO GROUP MEANS WITH UNEQUAL NUMBERS OF REPLICATIONS, AND THIS REVISED METHOD WAS USED FOR THIS WORK.

TECHNIQUES

Approximately 320 white leghorn fowls were secured from the poultry plant of the Virginia Agricultural Experiment Station, Blacksburg, Virginia, for this work. These birds were subdivided into groups of approximately 40 birds per group, and were maintained in 8 separate one-room range houses provided by the Poultry Husbandry Department. The data were obtained only from well feathered hens.

As this work required all levels of louse infestations on the birds, different degrees of infestation were maintained in the different groups. If a group of chickens built up an infestation larger than desired, the entire group was deloused by dusting the litter lightly with 4% malathion dust. When the insecticide was no longer effective (approximately one week), infested cocks were introduced to reinfest the hens. The hens were utilized immediately when they reached the desired infestation, that is, the appropriate position in the scale of coding systems.

For Coding Lice:

Four systems of coding populations of lice on chickens were used for this work. Each chicken was held in an upright position with one hand and the feathers were gently ruffled with the other hand. In this way a visual inspection was made to determine the degree of infestation of lice.

A. THE 8 POINT SYSTEM CITED BY EDGAR, ET AL.:

CODE	CITED MEANING	INTERPRETATION USED IN THIS PAPER
(0)	NO VISIBLE LICE	NO LICE SEEN DURING VISUAL INSPECTION
(1)	VERY LIGHT INFESTATION	1 OR 2 LICE SEEN DURING VISUAL INSPECTION
(2)	LIGHT INFESTATION	LICE DIFFICULT TO FIND
(3)	MED. LIGHT INFESTATION	LICE EASILY SEEN BUT THINLY SCATTERED
(4)	MED. INFESTATION	LICE MORE HEAVILY SCATTERED
(5)	MED. HEAVY INFESTATION	LICE NUMEROUS; MANY SHAFTS OF FEATHERS MAY HAVE SEVERAL LICE PRESENT
(6)	HEAVY INFESTATION	LICE VERY NUMEROUS; MOST SHAFTS OF FEATHERS APPEAR TO HAVE ONE TO SEVERAL LICE PRESENT
(7)	VERY HEAVY INFESTATION	EVERY SHAFT OF FEATHERS APPEARS TO HAVE SEVERAL TO MANY LICE PRESENT

B. THIS SYSTEM, DEVELOPED BY THE AUTHOR, CONSISTS OF COUNTING FOR A PERIOD OF EXACTLY ONE MINUTE UNDER THE ASSUMPTION THAT THE NUMBER OF LICE COUNTED WOULD BE DIRECTLY PROPORTIONAL TO THE NUMBER PRESENT.

A DIFFERENT CODE IS ASSOCIATED WITH EACH 25 LICE:

CODE	INTERPRETATION
(0)	NO LICE SEEN DURING A ONE MINUTE INTERVAL
(1)	1-25 LICE SEEN DURING A ONE MINUTE INTERVAL
(2)	26-50 LICE SEEN DURING A ONE MINUTE INTERVAL
(3)	51-75 LICE SEEN DURING A ONE MINUTE INTERVAL
(4)	76-100 LICE SEEN DURING A ONE MINUTE INTERVAL
(5)	101-125 LICE SEEN DURING A ONE MINUTE INTERVAL

C. The 4 point system cited by Telford:

CODE	CITED MEANING	INTERPRETATION USED IN THIS PAPER
(0)	NO VISIBLE LICE	NO LICE SEEN DURING VISUAL INSPECTION
(1)	1 TO APPROX. 100 LICE	ROUGHLY ESTIMATE A RANGE OF 1-100 LICE
(2)	PROPORTIONALLY MORE	ROUGHLY ESTIMATE A RANGE OF 101-1000 LICE
(3)	PROPORTIONALLY MORE	ROUGHLY ESTIMATE A POPULATION OF MORE THAN 1000 LICE

D. The 4 point system cited by Raffensperger:

(0)	NO VISIBLE LICE	NO LICE SEEN DURING VISUAL INSPECTION
(1)	VERY DIFFICULT TO FIND	1 OR 2 LICE SEEN DURING VISUAL INSPECTION
(2)	LIGHT TO MED. INFESTATION	FROM 3 TO RATHER NUMEROUS OR PLENTIFUL
(3)	MED. TO HEAVY INFESTATION	VERY PLENTIFUL

For Removing Lice:

Several techniques cited in the literature, variations thereof, and many independent ideas were tried in order to establish the most applicable method of removing total populations of lice from chickens.

Originally, the method cited by Beer and Cook (1957) was tried. This consisted of skinning the bird and placing the skin with the attached feathers into a solution of hot KOH and the enzyme trypsin. Everything except any lice which were present was supposed to be digested. The

THE LICE COULD THEN BE STRAINED OUT OF THE SOLUTION FOR COUNTING. HOWEVER, DIFFICULTY WAS ENCOUNTERED IN GETTING ALL OF THE SKIN AND FEATHERS OF A BIRD AS LARGE AS A CHICKEN DIGESTED. IT WAS FOUND, HOWEVER, THAT IF THE CHICKEN WAS PLUCKED, THE FEATHERS COULD BE COMPLETELY DIGESTED. THIS PROCEDURE REQUIRED SEVERAL DAYS FOR EACH FOWL, WHICH WAS MORE TIME THAN WAS AVAILABLE, SO A MORE RAPID METHOD WAS SOUGHT.

THE METHOD CITED BY FLOYD AND TOWER (1956) WAS TRIED, AS IT APPEARED TO BE LESS TIME CONSUMING. THIS METHOD CONSISTED OF HOLDING A CHICKEN FIRMLY OVER A LARGE PIECE OF SMOOTH PAPER. SEVERAL PINCHES OF PYRETHRUM DUST, WHICH IRRITATED THE LICE PRESENT, WERE DUSTED INTO THE FEATHERS. THIS CAUSED THE LICE TO MIGRATE TO THE OUTSIDE OF THE FEATHERS WHERE THEY COULD BE FLUFFED TO THE PAPER BY THE HAND. THEY COULD THEN BE COLLECTED AND COUNTED. IN THE ACTUAL TRIAL OF THIS METHOD, BOTH PYRETHRUM AND DIPTEREX WERE USED. IT WAS FOUND THAT THE LICE TENDED TO RESIST BEING BRUSHED FROM THE FEATHERS EVEN THOUGH THEY WERE MOVING TO THE OUTSIDE OF THE FEATHERS, AND THAT SOME OF THEM WOULD CRAWL ON THE HAND AND ARM OF THE PERSON DOING THE BRUSHING. MANY OF THE LICE KNOCKED ON THE PAPER IMMEDIATELY BEGAN MIGRATING TO ITS EDGE. IT WAS VERY DIFFICULT TO BRUSH THE LIVE LICE FROM THE PAPER EVEN IF THEY WERE INACTIVE, BECAUSE THEY TENDED TO CLING TO THE

FIBERS OF THE PAPER. DEAD LICE DO NOT EXHIBIT THIS TENDENCY. THE METHOD CITED BY DUNN (1932), OF APPLYING CHLOROFORM TO A TOWEL DRAPED AROUND THE CHICKEN TO ANESTHETIZE THE LICE PRESENT, WAS ALSO TRIED. CHLOROFORM, HOWEVER, DID NOT KEEP THE LICE IN AN ANESTHETIZED CONDITION LONG ENOUGH AFTER APPLICATIONS THAT RANGED UP TO SIX MINUTES.

IT WAS THOUGHT THAT AN EFFECTIVE METHOD OF REMOVING LICE FROM THE BODY OF CHICKENS MIGHT BE WITH A VACUUM CLEANER. A COMMERCIAL TYPE VACUUM CLEANER WAS SECURED AND THE DUST BAG REPLACED WITH A 350 MESH PLANKTON NET TO CATCH THE LICE. EACH CHICKEN WAS PLACED IN A POLY-ETHYLENE BAG WITH A WAD OF COTTON SATURATED WITH CHLOROFORM. THE NECK OF THE BAG WAS SEALED SNUGLY AROUND THE NECK OF THE CHICKEN IN SUCH A WAY AS TO LEAVE THE HEAD OF THE CHICKEN EXPOSED TO THE AIR. AFTER APPROXIMATELY 6 MINUTES, THE CHICKEN WAS REMOVED AND VACUUMED. IT WAS FOUND THAT THE LICE WERE RATHER EASILY REMOVED FROM THE BIRD IN THIS FASHION WHILE THEY WERE STILL ANESTHETIZED, BUT, AFTER APPROXIMATELY A MINUTE OF EXPOSURE TO THE AIR, THEY BEGAN TO BECOME ACTIVE AND REMOVAL DIFFICULT. BECAUSE OF THEIR ACTIVITY THEY WERE ALSO DIFFICULT TO REMOVE FROM THE PLANKTON NET. A DIFFERENT APPROACH TO THE SAME TECHNIQUE INVOLVED PLACING AN ASPIRATOR TYPE SUCTION JAR PARTIALLY FILLED WITH ALCOHOL BETWEEN THE VACUUM CLEANER AND THE NOZZLE, THUS CATCHING THE LICE IN THE ALCOHOL. HOWEVER, THE SMALLER SIZE OF THE TUBES GOING INTO THE SUCTION BOTTLE IN RELATION

TO THE DIAMETER OF THE VACUUM CLEANER TUBE, AND THE BUBBLING OF THE LICE THROUGH THE ALCOHOL, SUFFICIENTLY REDUCED THE SUCTION TO MAKE IT IMPOSSIBLE TO EASILY REMOVE ALL THE LICE FROM A CHICKEN.

ANOTHER ATTEMPT TO REMOVE LICE FROM CHICKENS WAS BASED ON THE HYPOTHESIS THAT LICE WOULD MIGRATE FROM A COOLING OBJECT TO A WARMER OBJECT. A BOTTLE OF WATER WAS HEATED TO THE NORMAL BODY TEMPERATURE OF CHICKENS (APPROXIMATELY 105°C.), WRAPPED WITH COTTON, AND PLACED BESIDE AN INFESTED CHICKEN WHICH HAD RECENTLY BEEN KILLED. MOST OF THE LICE LOCATED ON FEATHERS ADJACENT TO THE HEATED BOTTLE MOVED OUT TO THE ENDS OF THE FEATHERS, BUT ONLY A FEW MIGRATED TO THE COTTON. THOSE LICE SEVERAL INCHES FROM THE HEATED BOTTLE DID NOT SEEM TO SENSE THE HEAT.

ANOTHER METHOD THAT WAS TRIED IN THE SEARCH FOR THE MOST EFFECTIVE WAY TO REMOVE LICE FROM CHICKENS INVOLVED THE FOLLOWING PROCEDURE: AN INFESTED CHICKEN WAS KILLED AND PLACED IN A FREEZER AT A TEMPERATURE OF -27°C. FOR SEVERAL HOURS TO KILL THE LICE. THE CHICKEN WAS THEN REMOVED AND FLUFFED IN AN ORDINARY PAPER BAG. THE DIFFICULTIES ENCOUNTERED HERE WERE THAT THERE WERE TOO MANY PLACES IN AN ORDINARY PAPER BAG THAT WOULD CATCH AND HOLD THE LICE AND THAT A CHICKEN IN THE FROZEN STATE IS UNMANEUVERABLE, AND IF ALLOWED TO THAW THE FEATHERS FLUFF OUT OF THEIR FOLLICLES.

HAVING FOUND THAT THE LICE ARE MOST EASILY REMOVED WHEN DEAD AND THAT THE BEST WAY TO KILL THEM WAS BY FUMIGATION, IT WAS DECIDED TO FUMIGATE A CHICKEN AND TRY TO REMOVE THE LICE BY FLUFFING THE FEATHERS. A HEAVILY INFESTED CHICKEN, AFTER BEING FUMIGATED, WAS FLUFFED FIVE TIMES FOR FIVE MINUTE INTERVALS AND EACH SAMPLE SAVED SEPARATELY. WHEN COUNTED, IT WAS FOUND THAT OF ALL THE LICE COLLECTED, 71% CAME FROM THE FIRST 5 MINUTE INTERVAL, 18% CAME FROM THE SECOND 5 MINUTE INTERVAL, 7% CAME FROM THE THIRD, 3% FROM THE FOURTH, AND 2% FROM THE FIFTH. AS NEARLY 90% OF THE LICE CAME FROM THE FIRST 10 MINUTES OF FLUFFING, IT WAS CONSIDERED THAT 10 MINUTES WOULD BE A SUFFICIENT LENGTH OF TIME TO FLUFF THE BIRDS AND IF ALL BIRDS WERE TREATED UNIFORMLY THE RESULTS WOULD BE UNIFORM. THE TECHNIQUE FOR COLLECTING THE POPULATION OF LICE FROM CHICKENS THAT WAS FINALLY USED IS AS FOLLOWS: THE FEET OF EACH BIRD WERE SECURED TOGETHER. THE BIRDS WERE THEN PLACED IN INDIVIDUAL FUMIGATION CHAMBERS WHICH WERE ROUND OBLONG CARDBOARD BOXES THAT INSECTICIDE COMPANIES USED TO SHIP GALLON JUGS OF INSECTICIDE. SCREENWIRE CONTAINERS, 4-5 INCHES LONG AND 1-1 1/2 INCHES IN DIAMETER, WERE FILLED WITH COTTON AND SATURATED WITH METHYL BROMIDE (AN INEXPENSIVE, RELATIVELY SAFE FUMIGANT). ONE CONTAINER WAS PLACED IN THE FUMIGATION CHAMBER WITH EACH CHICKEN. THE TOP OF THE CHAMBER WAS LINED WITH PAPER TOWELS TO MAKE A TIGHTER SEAL AND THE LID

PUT IN PLACE. AFTER LEAVING THE CHICKENS IN THE CHAMBER FOR A MINIMUM OF THREE HOURS THEY WERE REMOVED AND HUNG BY THE FEET FROM A HOOK WHICH WAS SUSPENDED FROM THE CEILING BY A ROPE. THE WINGS OF THE CHICKEN WERE FASTENED OVER ITS BACK. A 12" x 23" POLYETHYLENE BAG, WHICH WAS PUNCTURED IN THE BOTTOM AND THREADED UP THE ROPE, WAS PULLED DOWN OVER THE CHICKEN. THIS FORCED THE LICE TO FALL STRAIGHT DOWN AS THEY WERE FLUFFED FROM THE CHICKEN, TO THE 4 FOOT X 4 FOOT PIECE OF SMOOTH PAPER LYING HORIZONTALLY ABOUT 3 INCHES BELOW THE HEAD OF THE CHICKEN. EACH CHICKEN WAS FLUFFED FOR TWO FIVE MINUTE INTERVALS BY PLACING THE HAND UNDER THE BAG AND RUFFLING THE FEATHERS. AFTER EACH FIVE MINUTE INTERVAL, TO AVOID GETTING TOO LARGE A PILE OF FEATHERS AND DEBRIS, THE PAPER WAS EMPTIED INTO A SMALL GLASS BOTTLE THROUGH A FUNNEL. THE COMPLETE SAMPLE FROM EACH CHICKEN WAS PLACED IN THE SAME BOTTLE, STOPPERED AND LABELED.

THIS TECHNIQUE ALLOWED SAMPLES TO BE TAKEN FROM A LARGE NUMBER OF BIRDS IN A DAY BECAUSE SEVERAL FUMIGATION CHAMBERS COULD BE USED SIMULTANEOUSLY. THE FLUFFING PROCEDURE TOOK APPROXIMATELY 15 MINUTES PER BIRD.

For Counting Lice:

PART OF THE METHOD CITED BY FLOYD AND TOWER (1956) FOR PREPARING SAMPLES OF LICE FOR COUNTING WAS TRIED. THIS WAS TO STIR THE SAMPLE INTO A BEAKER CONTAINING A 100 cc.

MIXTURE OF WATER AND A WETTING AGENT AND THEN POUR THIS INTO A SUCTION FILTER. THIS WOULD DISTRIBUTE THE LICE EVENLY ON THE FILTER PAPER. THE PART OF THE METHOD NOT TRIED WAS TO PLACE THE FILTER PAPER ON A SLIDE MOUNT RINGER AND COVER IT WITH A PIECE OF GLASS MARKED INTO 5 CONCENTRIC CIRCLES WITH BLACK RADIOMAT LANTERN TAPE. THE SLIDE MOUNT RINGER WAS THEN PLACED UNDER A STEREOMICROSCOPE AND EACH CIRCLE COUNTED SEPARATELY. FOR THIS WORK, THE FILTER PAPER ITSELF WAS MARKED OFF INTO 1/2 INCH SQUARES AND EACH SQUARE COUNTED UNDER THE STEREOMICROSCOPE. HOWEVER, AS THE SAMPLES ALWAYS CONTAINED A CERTAIN AMOUNT OF DEBRIS, IT WAS ALMOST IMPOSSIBLE TO MAKE AN ACCURATE COUNT FROM A DRY FILTER PAPER. TO MAKE COUNTING EASIER THE BOTTOM OF A PETRI DISH WAS MARKED OFF INTO 5/8 INCH SQUARES WITH A WAX PENCIL AND ALCOHOL ADDED. THE FILTER PAPER WAS PLACED INTO THE PETRI DISH AND THE LICE SCRAPED OFF WITH A SCALPEL. THE PAPER WAS THEN EXAMINED TO DETERMINE IF ANY LICE HAD BEEN MISSED. THE LICE IN THE DISH COULD BE COUNTED MUCH EASIER, BUT MANY HAD BEEN BADLY MUTILATED FROM BEING SCRAPED FROM THE FILTER PAPER. ALCOHOL ALSO SLOWLY DISSOLVES WAX PENCIL. TO OVERCOME THESE DIFFICULTIES, IT WAS DECIDED TO ALTER THE TECHNIQUE BY ADDING ALCOHOL DIRECTLY TO THE SAMPLE. PORTIONS OF THE SAMPLE WERE THEN DRAWN OFF WITH A MEDICINE DROPPER AND DEPOSITED IN A SPOT PLATE FOR COUNTING. THIS WORKED VERY WELL, BUT TOOK CONSIDERABLE TIME. IT WAS FINALLY DECIDED

TO DEPOSIT ALL OR PART OF THE DRY SAMPLE (DEPENDING ON THE AMOUNT OF BULK) INTO A PETRI DISH THAT WAS MARKED OFF INTO 5/8 INCH SQUARES WITH INDIA INK. ENOUGH ZYLENE WAS ADDED TO WET THE SAMPLE AND THEN HEAVY MINIERAL OIL WAS ADDED UNTIL THERE WAS A THIN LAYER OF THE VISCOUS MATERIAL AND LICE OVER THE BOTTOM OF THE PETRI DISH. THE PETRI DISH WAS PLACED ON THE STAGE OF A STEREOMICROSCOPE AND THE LICE WERE COUNTED USING 15 POWER MAGNIFICATION. A MECHANICAL COUNTER WAS USED IN MAKING THE COUNTS. THE SAMPLE BOTTLE WAS EXAMINED ALSO TO DETERMINE THE NUMBER OF LICE THAT WERE MISSED. AFTER EACH COUNT, THE PETRI DISH WAS CLEANED WITH VERY SOFT TISSUE PAPER, AS THIS WOULD NOT REMOVE THE INDIA INK MARKINGS.

For Statistical Analysis:

THE DATA WERE SUBJECTED TO SEVERAL STATISTICAL TESTS. ONE SUCH TEST DETERMINED THE AMOUNT OF CORRELATION BETWEEN THE NUMERICAL SUBDIVISIONS WITHIN EACH CODING SYSTEM AND THE ACTUAL NUMBERS OF LICE THEY WERE ASSUMED TO REPRESENT. A SECOND TEST, BASED ON THE AMOUNT OF CORRELATION, DETERMINED IF ALL SYSTEMS WERE EQUALLY GOOD OR IF ANY WERE SIGNIFICANTLY DIFFERENT FROM THE OTHERS. AN ANALYSIS OF VARIANCE WAS PERFORMED TO DETERMINE IF THE DATA WITHIN EACH SYSTEM WOULD FOLLOW A LINEAR REGRESSION LINE. A SECOND ANALYSIS OF VARIANCE DETERMINED IF THE MEANS WITHIN EACH SYSTEM WERE

EQUAL. IN THOSE CODING SYSTEMS IN WHICH SOME OF THE MEANS WERE FOUND TO BE UNEQUAL, A MULTIPLE RANGE TEST WAS PERFORMED TO DETERMINE IF ANY OF THE MEANS WITHIN THAT SYSTEM WERE EQUAL. TO DETERMINE IF THE AMOUNT OF OVERLAPPING WITHIN EACH SYSTEM WAS SIGNIFICANT, A BARTLETT'S TEST FOR HOMOGENITY OF VARIANCES WAS PERFORMED.

RESULTS

After the louse populations on 79 female chickens were coded by the four coding systems already described, they were collected and counted. The numbers of lice from all chickens are listed below in their appropriate places in each of the four systems.

TABLE A

Actual Louse Counts for System A

0	1	2	3	4	5	6	7
3	6	24	74	264	425	766	2479
14	9	29	120	453	588	1200	3213
17	14	49	128	654	1036	1209	3308
19	17	53	316	861	1204	1314	3829
33	17	84	580	924	1212	1329	3903
48	29	113	793	936	1368	1518	5200
	30	130	1205	981	1377	2066	5774
	31	208		1026	1417	2196	
	33	238		1092	1608	2222	
	43			1336	1902	2250	
	46				2026	2597	
	59				2544	2730	
					2798	2845	
						3211	
						3872	
134	330	928	3216	8527	19505	31325	27706

TABLE B

ACTUAL LOUSE COUNTS FOR SYSTEM B

0	1	2	3	4	5
14	3	264	425	766	2479
17	6	453	588	1200	3213
19	9	580	861	1204	3308
48	14	654	924	1314	3829
	17	793	981	1368	3872
	17	936	1209	1518	3903
	24	1026	1377	1608	5200
	29	1036	1902	2196	5774
	29	1092	2026	2222	
	30	1212	2066	2250	
	31	1329	2544	2730	
	33	1336	2597	2798	
	33	1417		2845	
	43			3211	
	46				
	49				
	53				
	55				
	74				
	84				
	113				
	120				
	128				
	130				
	208				
	238				
	316				
	1205				
98	3137	12128	17500	27230	31578

TABLE C

Actual Louse Counts for System C

0	1	2	3
3	6	425	2479
14	9	588	3213
17	14	654	3308
19	17	766	3829
33	17	861	3903
48	24	936	5200
	29	1036	5774
	29	1092	
	30	1200	
	31	1204	
	33	1209	
	43	1212	
	46	1314	
	49	1329	
	53	1368	
	55	1377	
	74	1417	
	84	1518	
	113	1608	
	120	1902	
	128	2026	
	130	2066	
	208	2196	
	238	2222	
	264	2250	
	316	2544	
	453	2597	
	580	2730	
	793	2798	
	924	2845	
	981	3211	
	1026	3872	
	1205		
	1336		
134	9458	54373	27706

TABLE D

Actual Louse Counts for System D

0	1	2	3
3	6	24	425
14	9	29	588
17	14	49	766
19	17	53	1036
33	17	74	1200
48	29	84	1204
	30	113	1209
	31	120	1212
	33	128	1314
	43	130	1329
	46	208	1368
	55	238	1377
		264	1417
		316	1518
		453	1608
		580	1902
		654	2026
		793	2066
		861	2196
		924	2222
		936	2250
		981	2479
		1026	2544
		1092	2597
		1205	2730
		1336	2798
			2845
			3211
			3213
			3308
			3829
			3872
			3903
			5200
			5774
134	330	12671	78536

The means of each subgroup for the four coding systems are listed in Table E.

TABLE E

The Means of the Subgroups for the Four Coding Systems

System	0	1	2	3	4	5	6	7
A	22	28	103	459	853	1500	2088	3958
B	25	112	933	1458	1945	3947		
C	22	278	1699	3958				
D	22	28	487	2243				

An analysis of variance indicated that there was a significant difference in the means of the subgroups within each coding system. The results of a multiple range test to determine which of the means of the subgroups were different are given in Table E. Those means which are underlined by the same straight line are not significantly different at the .05 level.

A correlation between the scores and the actual louse count was performed and the results are listed under r (correlation coefficient) in Table F for each coding system. A test showed that the correlation coefficient for coding system D was significantly lower than the correlation coefficients for the other systems at the .05 level.

TABLE F

CORRELATION COEFFICIENTS FOR THE CODING SYSTEMS

SYSTEM	R	R^1
A	.82	.87
B	.86	.87
C	.81	.85
D	.68	

THE SUBGROUPS WITHIN CODING SYSTEMS A, B, AND C WHICH WERE NOT SIGNIFICANTLY DIFFERENT FROM EACH OTHER WERE COMBINED AND A NEW CORRELATION COEFFICIENT WAS CALCULATED FOR THEM. THESE NEW CORRELATION COEFFICIENTS ARE LISTED UNDER R^1 IN TABLE F.

SUMMARY AND CONCLUSIONS

THE PRIMARY OBJECTIVE OF THIS WORK WAS THE EVALUATION OF SEVERAL CODING SYSTEMS USED TO ESTIMATE THE DEGREE OF LOUSE INFESTATION ON CHICKENS. ON THE BASIS OF THE DATA COLLECTED AND STATISTICAL TESTS PERFORMED, WE NOW SUMMARIZE OUR CONCLUSIONS.

IT WAS FOUND THAT THE LINEAR RELATIONSHIP BETWEEN THE SUBGROUPS OF THE CODING SYSTEMS AND THE ACTUAL NUMBERS OF LICE, WHICH HAD HERETOFORE BEEN ASSUMED, ACTUALLY EXISTED.

THIS WORK ALSO INDICATED WHICH OF THE FOUR SYSTEMS TESTED WERE PROBABLY THE BEST CODING SYSTEMS. SYSTEMS A, B, AND C WERE CONSIDERED SUPERIOR TO SYSTEM D BECAUSE THE SCORES FOR A, B, AND C WHEN CORRELATED WITH THE ACTUAL COUNTS RESULTED IN SIGNIFICANTLY HIGHER CORRELATION COEFFICIENTS THAN SYSTEM D. SINCE SEVERAL POINTS IN SYSTEM A WERE NOT SIGNIFICANTLY DIFFERENT, SYSTEM A WAS CONSIDERED INFERIOR TO SYSTEMS B AND C. SYSTEM B COULD BE CONSIDERED SUPERIOR TO SYSTEM C BECAUSE THE METHOD OF DETERMINING THE SUBGROUP THAT REPRESENTS EACH POPULATION OF LICE MOST ADEQUATELY IS LESS ARBITRARY AND BECAUSE THERE ARE MORE SUBGROUPS. SYSTEM C, ON THE OTHER HAND, IS MUCH FASTER TO USE.

IT WAS FOUND THAT WHEN THE POINTS IN SYSTEMS A, B, AND C WHICH WERE NOT SIFNIFICANTLY DIFFERENT WERE COMBINED, THERE WAS A SLIGHTLY HIGHER CORRELATION (R^1 IN TABLE F).

A SECOND OBJECTIVE OF THIS WORK WAS TO DEVELOP A PRACTICAL TECHNIQUE FOR REMOVING AND COUNTING THE TOTAL LOUSE POPULATION. THE TECHNIQUE FOR DOING THIS, WHICH WAS FOUND TO WORK WELL IN THIS CIRCUMSTANCE, HAS BEEN DISCUSSED.

IT BECAME APPARENT AFTER THIS WORK WAS BEGUN THAT, DUE TO LIMITATIONS OF TIME AND FACILITIES, THERE WOULD BE SOME QUESTIONS THAT COULD NOT BE ANSWERED. IT WAS IMPRACTICABLE, FOR EXAMPLE, TO DUPLICATE THIS STUDY USING DIFFERENT BREEDS OF CHICKENS OR DIFFERENT SPECIES OF LICE WHICH INFEST CHICKENS. HOWEVER, IT IS FELT THAT THIS STUDY WAS A DEFINITE STRIDE FORWARD, AS IT TESTIFIED TO THE MERIT OF USING CODING SYSTEMS AS RAPID AND SIMPLE METHODS FOR ESTIMATING LOUSE POPULATIONS ON CHICKENS.

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ABSTRACT

An evaluation of several coding systems, or arbitrary sets of numbers, used to estimate louse populations on chickens was performed. Included in the study was the development of a technique for the removal of lice from chickens and a technique for making actual louse counts.

The procedure involved consisted of coding, by 4 coding systems, the louse infestations on 79 individual chicken hens. Each chicken was then fumigated and the feathers fluffed to remove the lice after which counts were made using a stereomicroscope. The resulting numbers were subjected to several statistical tests which indicated that there are linear relationships between the numerical symbols and the infestations they arbitrarily represent. It was also found that due to the improper spacing of values, the accuracy of one coding system was inferior to the other three systems.