THE EFFECT OF CAGE DENSITY, TRANSPORTING OF PULLETS AND VACCINATION AGAINST MAREK'S DISEASE ON THE PERFORMANCE OF S. C. W. L. PULLETS

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY
HUGH CHARLES GOAN

1971

This is to certify that the

thesis entitled

THE EFFECT OF CAGE DENSITY, TRANSPORTING OF PULLETS AND VACCINATION AGAINST MAREK'S DISEASE ON THE PERFORMANCE OF S.C.W.L. PULLETS

presented by

Hugh Charles Goan

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Poultry Science

O.C. Sheppard
Major professor

Date October 25, 1971

O-7639

ABSTRACT

THE EFFECT OF CAGE DENSITY, TRANSPORTING OF PULLETS AND VACCINATION AGAINST MAREK'S DISEASE ON THE PERFORMANCE OF S.C.W.L. PULLETS

By

Hugh Charles Goan

The herpesvirus of turkeys (HVT strain FC 126) was used to vaccinate 700 chicks at a dose level of 9,800 plaque forming units per chick. An additional 700 chicks were not vaccinated. The chicks were reared to 20 weeks of age in a start-grow-lay cage system. The vaccinated chicks were not intermingled in the same cage, but some of each group were located in adjacent cages due to randomization procedures.

Significant differences ($P<0.01$) were found for total mortality, Marek's disease mortality and feed consumed per bird between the vaccinated and unvaccinated pullets. Mortality caused by Marek's disease in the unvaccinated group was seven and one-half times greater than in the unvaccinated group. There was no difference in body weight between the two groups.

In the egg laying portion of the experiment, a 3 x 2 factorial experimental design was used. The three factors were HVT-vaccination, transporting of pullets and cage density. The birds could be in any treatment combination of vaccinated or unvaccinated, transported or not transported and eight birds/cage or 10 birds/cage. Only 432

vaccinated and 432 unvaccinated pullets from the growing period were used.

During the laying period, vaccination had a highly significant ($P<0.01$) effect on hen housed egg production, total mortality, Marek's disease mortality and lymphoid leukosis mortality. The vaccinated birds had a greater hen housed egg production, lower total mortality, lower Marek's disease mortality and lower lymphoid leukosis mortality than did the unvaccinated birds.

Egg production on a hen day basis was significantly ($P<0.05$) affected by density. Age at sexual maturity and Haugh unit scores (egg quality) were significantly affected by transporting the birds. The factors of vaccination, cage density, and transporting of pullets had no significant effect on percent blood spots, egg weight, egg shell thickness or body weight.

THE EFFECT OF CAGE DENSITY, TRANSPORTING OF PULLETS AND VACCINATION AGAINST MAREK'S DISEASE ON THE PERFORMANCE OF S.C.W.L. PULLETS

By

Hugh Charles Goan

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Poultry Science

1971

ACKNOWLEDGEMENT

The author wishes to acknowledge the assistance of and to express appreciation to:

Dr. C. C. Sheppard for assistance, counsel and guidance during the course of graduate study and in the planning of the research and preparation of the thesis.

Dr. H. C. Zindel and Dr. Theo H. Coleman for their assistance and supervision during the course of graduate study and in preparation of the thesis.

Dr. H. E. Larzelere and Dr. Mason Miller for serving on the graduate committee and for reviewing the thesis.

Dr. H. G. Purchase and the personnel at the U.S.D.A. Regional Poultry Research Laboratory for their assistance in this research.

Dr. J. L. Gill and Dr. L. R. Champion for their assistance in the analysis of the data.

My wife, Barbara, and son, Scott, for their patience, assistance and encouragement during the period of graduate study and research.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Marek's disease and Marek's disease vaccine

Marek's disease (MD) has been a problem for the poultry industry, for many years. It has been estimated that MD has caused losses in excess of $150 million per year to the poultryman (AAAP Report, 1967).

MD, in the past, has been referred to as neural lymphomatosis, fowl paralysis, range paralysis and acute leukosis. A DNA herpes-type virus appears to be the causative agent which induces lymphoid accumulations in the nervous system, various visceral organs, eye, skin and muscle.

MD usually affects growing pullets between the ages of six and 18 weeks, but can also affect older birds. There are problems in identifying MD tumors due to their similarity in gross and microscopic appearance to lymphoid leukosis. Also lymphoid leukosis usually appears in a flock of chickens when they are 16 weeks of age and older, at which time MD is very prevalent.

Attempts to control MD in commercial flocks by sanitation and isolation rearing has met with limited success. During the past two years, MD vaccines have been developed. These vaccines should help the poultryman in his struggle with the MD problem.

One vaccine was developed in England but at this time has not been approved for use in the United States. Another MD vaccine was developed in the United States by researchers at the USDA Regional

Poultry Research Laboratory, East Lansing, Michigan. This vaccine was developed from a herpevirus of turkeys (HVT) that was isolated from turkey blood. In laboratory experiments, HVT has been found to offer protection to growing chicks against MD. As yet, the long term effects of HVT on the laying bird are not known.

The experiment discussed in this thesis was conducted in cooperation with the USDA Regional Poultry Research Laboratory, East Lansing, Michigan. The experiment was a part of the first field trials that were designed to study the effect of the HVT vaccine on the growing and laying chicken.

Cage density

Changes are constantly being made in equipment used by the poultryman. The size and design of the cage varies among the many equipment companies. Often the poultryman will put five birds in a cage which was designed to hold four birds. This practice allows the cost of the equipment to be spread over a greater number of birds. Approximately 50 square inches per bird is the standard now being used by the commercial poultry industry.

There are management problems associated with increasing the population in the cage. Cannibalism, limited feeder space, limited water space, a higher percent cracked eggs and/or ventilation problems may result. Often in cages that are 20 or more inches in depth, birds that are crowded toward the rear of the cage have a difficult time reaching the feed and water.

Transporting pullets

Many commercial poultrymen have speculated that stresses such as high temperatures, freezing temperatures, debeaking, catching and transporting pullets have some effect on stimulating an outbreak of MD. They base their statements on experiences they have incurred with their flocks.

Pullets, at times are transported during the daytime when ambient temperatures are greater than 90°F. These pullets are usually crowded into the coops and whenever the truck is stopped for any period of time there is very little air moving across the birds. The combination of high temperature and little air movement can result in severe stress and sometimes death from heat exhaustion. Conversely, during the winter pullets are sometimes transported during freezing weather and are subject to frostbite which could have an effect on subsequent egg production. Also, the physical handling of the pullets while catching, cooping, loading, unloading, and putting them in cages could possibly have an effect on the subsequent performance of the pullet.

An answer to cooping and hauling that has been advanced is the use of a start-grow-lay cage system. In this type system, one day-old chicks are put into the cages. They would remain there throughout the growing and laying period. In addition to eliminating cooping and transporting, the costs involved in those processes would be eliminated.

Objectives

The objectives of this experiment were (1) to compare the performance of growing pullets that were vaccinated against MD with the performance of unvaccinated pullets, and (2) to determine the effects of vaccination against MD, cage density and transporting of birds on the performance of the laying hen.

REVIEW OF LITERATURE

Development of a Marek's disease vaccine

The initial description of Marek's disease (MD) was made by Marek (1907). Junhgerr (1939), Andrewes and Glover (1939), and Wight (1963) mentioned that several species of birds may be natural hosts for MD. These various species include the chicken, turkey, pheasant, quail, pigeon, duck, goose, canary, budgerigar and swan. Of those fowl, the identification and control of MD in the chicken has been of the greatest interest to the researcher.

According to Calnek and Witter (1971), the clinical sign associated with MD is usually complete paralysis of one or more of the extremities. The loss of nervous control may affect the wings, neck and especially, the legs. Blindness may result from MD involvement of the iris. Other signs are weight loss, paleness, anorexia and diarrhea. They suggest that under commercial poultry conditions, death of the birds often result from starvation and dehydration due to the inability to reach food and water. Also mentioned by Calnek and Witter were areas where MD tumors have been found to develop. These tumors have been found in the ovary, testis, heart, lung, mesentery, kidney, liver, spleen, adrenal gland, pancreas, proventriculus, intestine, iris, skeletal muscle and skin.

Siccardi and Burmester (1970) mentioned that MD occur at any age after about six weeks but most frequently between eight and 24 weeks of age. They added that mortality caused by lymphoid leukosis usually starts when birds are about 16 weeks of age.

MD was reported to be easily transmitted by direct or indirect contact of birds by Biggs and Payne (1963). Further research by Biggs and Payne (1964) and Witter et al. (1966) confirmed the idea that MD and lymphoid leukosis were etiologically unrelated. However, the similar pathologic features of the two diseases continue to create diagnostic problems.

Churchill and Biggs (1967) presented the first evidence that MD was caused by a herpesvirus. That report was later confirmed in the evidence presented by Nazerian et al. (1968), Solomon et al. (1968), Witter et al. (1969) and Calnek et al. (1970).

MD antigens were found consistently in the superficial layers of the feather follicle epithelium by Calnek and Hitchner (1969). They concluded that the feather follicle seemed to be a site in which viral replication proceeded to completion. Beasley et al. (1970) found that dander and dust from infected birds carried the MD virus.

The first report that chickens could be immunized against MD was provided by Churchill et al. (1969). The vaccine was developed by using a live attenuated MD virus (HPRS-16) that was grown and passaged in cultured chicken kidney cells. Their results showed that the use of an attenuated MD virus would protect chickens against challenge with a virulent MD virus.

Four field trials were conducted by Biggs et al.(1970) in which the live attenuated MD virus (HPRS-16) was used to immunize chicks against MD. Approximately 19,000 chickens were involved in the trials. In two of the trials, one group of chicks was vaccinated at one day old and another group at 21 days old, while a third group was not vaccinated. The mortality in the vaccinated chicks was significantly lower than mortality in the unvaccinated chicks in one of the trials, but there was little difference in mortality for the other trial. In the other two trials, chicks were unvaccinated or were vaccinated at 21 days of age. There was significantly less mortality due to MD in the vaccinated chicks than in the unvaccinated chicks.

Two agents were used by Weston and Smith (1969) to vaccinate chicks against MD. These agents were (1) blood from an adult flock which had experienced high losses from a natural outbreak of MD and (2) a highly virulent agent of MD (H-IV). Neither agent was successful in reducing the incidence of MD.

Okazaki et al. (1970) evaluated HVT, strain FC 126, as a vaccine for protection against MD. One day old chicks were vaccinated, and the HVT vaccine gave protection against the development of MD. The vaccine was reported to be nonpathogenic and noncontagious. Seventeen field trials, involving approximately 130,000 birds, were conducted by Purchase et al. (1971). The HVT, strain FC 126, was used as a vaccine for protection against MD. The dosage level of vaccine administered in the trials ranged from 360 to 11,500 plaque forming units per chick. They found that dose levels of 2,000 and 5,000 plaque forming units per chick were equally effective in protecting against MD. In three of the trials, HVT gave 100 percent protection. Purchase and co-workers concluded that vaccination with HVT was a safe and effective method for controlling MD.

Edison and Anderson (1970) inoculated one-day old chicks with 3,000 to 5,000 plaque forming units of either HVT or an attenuated MD herpesvirus. The birds were protected against MD when challenged by natural or artificial exposure up to 37 weeks of age. They also studied the method of administering the MD vaccine. They reported that subcutaneous injections of the MD vaccine was more effective than intranasal or ocular administration.

Edison et al. (1970) conducted 17 field trials which involved 391,202 birds to evaluate various experimental vaccines for the control of MD. The vaccines evaluated were (1) attenuated MD herpesvirus, (2) HVT, strain FC 126, and (3) HVT, strain WHG. Their results showed that MD herpesvirus vaccine gave only partial protection to chicks vaccinated in the field trials, while both strains of HVT were found to offer a higher degree of protection against MD than did the MD herpesvirus.

Cage density

The laying hen, during the past 15 years, has been subjected to a more dense population in the laying cage. Depending upon the size of the cage. One, two, three, four, five and up to 25 laying hens may be housed in one cage.

Parker and Rogers (1954) found no difference in egg production or mortality for layers housed in individual or colony cages. The performance of individually caged layers was compared with the performance of colony caged layers (25 birds per 3 1/2'x 8' cage) by Schupe and Quisenberry (1961). The individually caged layers had significantly higher egg production and lower mortality than did the colony caged layers. Lowe and Heywang (1964) reported that hen day egg production was 10 percent greater for two birds per 12" x 18" cage than for five birds per 24" x 18" cage. They also stated that mortality tended to increase as the number of birds per cage increased.

Quisenberry and Bradley (1964) conducted an experiment in which there were one, two, three, five, seven or 10 birds per cage. They found egg production to be the highest with one bird per cage and the lowest with two or three birds per cage. There were no significant differences in body weight, egg size or mortality between the different groups.

Moore et al. (1965) found that the cost of producing a dozen eggs was less with two birds per 8" x 16" cage than with either three or four birds per 16" x 16" cage.

Bell and Little (1966) and Ruggles et al. (1967) housed two, three or four birds per 12" x 18" cage. Both groups found that egg production decreased significantly as the number of birds per cage increased. Both groups also reported that mortality tended to increase as the number of birds per cage increased.

Tower et al. (1967) reported on the performance of two, five, ten and 20 birds per cage at a density of .625 square feet per bird. A constant density was maintained by replacing the dead birds with a wire dummy. The ten and 20 birds per cage laid significantly less eggs per bird than did the two and five birds per cage. However, the ten birds per cage had the highest income per bird.

Owings et al. (1967) housed two and three birds per 10" x 16" cage and reported that the two birds per cage laid at a higher rate of production than did the three birds per cage.

Marr et al. (1967) conducted two experiments dealing with the evaluation of cage density for laying hens. In the first experiment, hens maintained at 128 and 80 square inches of space per bird produced 236 and 224 eggs on a hen-housed basis, respectively. The second experiment compared the performance of three birds per cage at 53 square inches per bird, two birds per cage at 64 square inches per bird, three birds per cage at 64 square inches per bird and two birds per cage at 80 square inches per bird. Hen housed egg production for the four groups was 178, 194, 189, and 203 eggs per bird, respectively. Also, percent mortality increased as the space per bird decreased.

Wilson et al. (1967) put one, two or three hens into 8" x 18" or 10" x 18" cages. Dead birds were replaced to maintain a constant density. Egg production per bird was significantly less with three than with one or two birds per cage. Egg weight and egg quality were not affected by cage density. They also found that as the number of birds per cage increased, mortality tended to increase.

Adams and Jackson (1968) compared the performance of two or four birds per 12" x 18" cage and eight or 16 birds per 28" x 30" cage. The two and four birds per cage laid significantly more eggs per bird and had significantly less mortality than did the eight and 16 birds per cage.

The performance of hens under five different cage regimes was evaluated by Champion and Zindel (1968). The five cage regimes were: (1) individually in 8" x 16" cages; (2) two birds in 8" x 16" cages; (3) three birds in 12" x 16" cages; (4) four birds in 16" x 16" cages;

and (5) six birds in 24" x 16" cages. They found that density had a significant effect on hen-housed egg production with the individually caged birds having the highest production. There were no significant differences in body weight or egg weight among the different regimes.

Ostrander and Young (1969) housed laying hens at two, three, four or five birds per 12" x 18" cage. Egg production per bird and feed efficiency were significantly lower for the five birds per cage than for the other groups. Over a two year test period, the four birds per cage proved to be the most profitable.

Three, experiments which involved cage density, were conducted by Wayman et al. (1969). In the first experiment, four, six, eight or 10 birds were placed in 24" x 24" cages. For the second experiment, one, two or three birds were housed in 12" x 18" cages. In the third experiment, three or four birds were placed in 12" x 18" cages. For each experiment no significant differences were observed for hen day or for hen housed egg production. In experiment one, the four birds per cage required significantly less feed per dozen eggs and the 10 birds per cage had significantly less body weight gain. For the three trials, feed consumption, mortality, egg weight and percent cracked eggs were not significantly affected by density.

Wildey (1969) reported no difference in egg production of pullets reared at the same cage density and housed at one or two birds per 8" x 16" cage during egg production. Mather and Gleaves (1970) housed two, four or six birds per 16" x 18" cage and reported that egg production decreased as the number of birds per cage increased.

Marr and Greene (1970) placed two, three, four, five, six or seven pullets per cage with comparable floor space per bird. They reported no significant difference in egg production or egg weight. In a second experiment, two birds were put into 8" x 16" cages while three birds were put into 10" x 18" cages. The two birds per cage produced significantly more eggs per bird than did the three birds per cage. They suggested that performance was affected more by space and shape of the cage in relation to capacity than by the number of birds per cage.

Marks et al. (1970) reported no significant difference or trends noted for egg production, egg weight, specific gravity or Haugh units for birds housed at densities of one or two birds per 10" x 18" cage and five birds per 18" x 20" cage. Fowler and Quisenberry (1970) placed three, four, six, nine, 10 or 15 birds per cage at a constant density of 48 square inches per bird. They found that egg production, egg weight, livability and body weight were superior for the three and four birds per cage when compared to the other groups.

Dorminey and Arscott (1971) compared the performance of two, three or four birds per 12" x 18" cage and reported lower egg production and feed efficiency as bird density increased; however, the difference was not significant. Also, there was no difference in body weight gain, egg weight, Haugh units, shell thickness, or the number of blood and meat spots due to density. They noticed a trend that percent cracked and percent body checked eggs increased as bird density increased.

Transporting pullets

Only two reports have been found which have dealt with the effects of transporting pullets on their subsequent egg production.

Under simulated hauling conditions, Welter et al. (1967) exposed

11 and 20 week old pullets to ambient temperatures of 44°F and 99°F for six and one-half hours. In trial one, the exposure of the pullets to the high or low temperatures, had no significant effect on egg production, body weight, age at sexual maturity, feed efficiency, egg quality, shell thickness, or laying house mortality. In trial two, egg shell thickness was significantly depressed in birds exposed to 44°F at 11 weeks of age and in birds exposed to 99°F at 20 weeks of age. Also, in trial two, the results showed a 29 eggs per hen difference in production which was reported as not being significantly different. The 29 eggs per hen was approximately 13 percent difference in production. The researchers concluded that growing pullets can be cooped and hauled without significantly affecting subsequent egg production or other economic factors.

Champion et al. (1966) reported on the use of an orally administered tranquilizer (Pacitran) prior to the catching, crating and transporting of started pullets. They reported that the treated pullets were less excitable during the catching and cooping procedures. They also found that the use of a tranquilizer had a beneficial effect, of a minor order, on egg production. Champion and co-workers then postulated that the beneficial effect of Pacitran might be related to social status, behavior, hierarchy and social tension. A further comment by Champion (1971) was that the tranquilizer might have, to some extent, had some effect on the stresses of catching, cooping and transporting the pullets.

MATERIALS AND METHODS

Growing period

On November 20, 1969, a total of 700 one-day-old commercial egg-type chicks were vaccinated intraabdominally with 9,800 plaque forming units of HVT, strain FC 126, per chick. The intraabdominal vaccination was done by Dr. H. G. Purchase of the U.S.D.A. Regional Poultry Research Laboratory. An additional 700 chicks were not vaccinated with HVT and are hereafter referred to as unvaccinated birds. The chicks were selected at random for the vaccinated and unvaccinated groups. The chicks were wingbanded as a means of identification, and then placed in a start-grow-lay cage system with 25 chicks per cage. The vaccinated and unvaccinated birds were not intermingled in the same cage but were, in a number of cases, located in adjacent cages as a result of the randomization procedures.

The experimental room was 23' x 40' and contained eight rows of cages with a total of 112 cages. The rows were in blocks of four in a modified stair-step design. The chicks were placed in the top 56 cages of the start-grow-lay system. At five weeks of age, one-half of the pullets were moved from the top rows of cages to the lower rows. Each cage was 24 inches wide, 22 inches deep and 16 inches high. The bottom was a 1" x 1" wire mesh. A removable false bottom, 1/2" x 1" wire mesh, was used during the first seven weeks and then removed. A 24" feeder was located on the front of each cage, but only

21 linear inches of feeder space were available to the chicks. This reduced feeder space was caused by the feeder hangers and water hanger. One Hart cup per cage provided water for the chicks.

The composition of the starting and growing ration is shown in Table 1. The rations were hand fed and were available ad libitum. The starting ration was fed during the first seven weeks and the growing ration during the remaining 13 weeks of the growing period.

An electric radiant heat panel system was the source of heat for the chicks. The heating panels were nailed to the ceiling directly above the top rows of cages. The temperature during the first week, at chick level, was approximately 85°F and was reduced 5°F per week until approximately 67°F was reached. The ventilation was by an 18 inch exhaust fan that moved 3,800 cubic feet of air per minute.

The chicks from day-old until four weeks of age received 24 hours of light daily. From four weeks until 14 weeks of age, the day-length was reduced to 14 hours. After 14 weeks of age, the day-length was reduced by two hours per week until an eight-hour light period per day was reached. The light was provided by 25-watt incandescent light bulbs that were controlled by a time clock and rheostat.

The pullets were vaccinated against Newcastle disease, infectious bronchitis, epidemic tremors and fowl pox during the growing period. The birds were debeaked at seven weeks of age.

At four weeks of age, 11 and 22 cockerels were removed from the vaccinated and unvaccinated group, respectively. Thus, the percent mortality will be based on the remaining number of pullets. At 20 weeks, all birds were handled and the cull birds were removed and killed.

Table 1. Composition of the chick starter and pullet grower diets.

Ingredient	Starter Diet (percent)	Grower Diet (percent)
Yellow Corn	52.95	39.95
Oats	5.00	30.00
Wheat Middlings	5.00	15.00
Alfalfa meal (17%)	4.00	2.50
Meat & bone scraps (50%)	2.50	2.50
Soybean meal (45%)	25.00	6.00
Fish meal (55%)	2.00	1.50
Dried whey	2.00	1.00
Ground limestone	.50	.50
Dicalcium Phosphate	.50	.50
Salt	.30	.30
Vitamin-trace mineral premix	.25	.25
Total	100.00	100.00

Table 2. Composition of the layer diet.

Ingredient	Layer Diet (percent)
Yellow Corn	65.24
Soybean meal (50%)	15.50
Alfalfa meal (17%)	3.00
Meat & bone meal (50%)	2.50
Fish meal (55%)	2.50
Dried whey	2.00
Limestone	6.00
Dicalcium phosphate	1.10
Salt	.30
Vitamin-mineral premix	.50
Choline chloride	.10
Zinc oxide	.01
Tallow, stabilizer	1.25
Total	100.00

A necropsy was performed on all birds which died during the 20 weeks, and on the culls. The necropsies were conducted by Dr. H. G. Purchase of the U.S.D.A. Regional Poultry Research Laboratory, East Lansing, Michigan. The procedures and diagnostic criteria have been described by Purchase et al. (1971).

Data were collected for mortality, feed consumption and 20 week body weights. The feed consumption and body weight data were analyzed by the analysis of variance (Snedecor, 1956). A Chi-square test was used for the analysis of the mortality data.

Laying period

The egg production portion of the experiment began April 10, 1970 at which time the pullets reached 20 weeks of age. The cages and equipment used during the growing period were also used during the laying period. Of the 611 vaccinated and 483 unvaccinated healthy pullets, only 432 from each group were needed for the egg production experiment.

The experimental design of the laying experiment was a 3 x 2 factorial plus replication. The three factors were HVT-vaccination, transporting and cage density. The birds could be in any treatment combination of vaccinated or unvaccinated, transported or not transported, eight birds/cage or 10 birds/cage. The eight treatment combinations were (1) vaccinated, transported, eight birds/cage; (2) vaccinated, transported, 10 birds/cage; (3) vaccinated, not transported, eight birds/cage; (4) vaccinated, not transported, 10 birds/cage; (5) unvaccinated, transported, eight birds/cage; (6) unvaccinated, transported, 10 birds/cage; (7) unvaccinated, not transported, eight birds/cage; and (8) unvaccinated, not transported, 10 birds/cage. Each treatment was replicated 12 times. The treatments having eight birds/cage or 10 birds/cage, had a total of 96 and 120 birds, respectively.

One-half (216) of the vaccinated and one-half (216) of the unvaccinated birds were transported. The procedures for transporting were similar to those used by the commerical poultry industry. The birds were caught at 6:00 P.M. and put into coops. Sixteen birds were put into each coop. The coops were stacked and remained in the experimental room until loading time. At 12:30 A.M. the coops were loaded on a one-half ton pick-up truck. Between the hours of 2:00 A.M. and 6:00 A.M. the birds were transported for a distance of approximately 175 miles. The speed of the truck, at times, reached and maintained a speed of 65 mph. During the transporting period, the average temperature at the Lansing, Michigan airport was 40°F.

At the end of the transporting period, the coops were removed from the truck and returned to the experimental room. The birds were not removed from the coops until 9:30 A.M.. When removed from the coops, the birds were returned to the start-grow-lay cages at a density of eight or 10 birds/cage.

The 432 birds that were not transported were placed at a desired density of eight or 10 birds/cage.

The floor space available to birds housed at eight per cage, was 66 square inches per bird, while the 10 birds per cage had 52.8 square inches per bird.

The laying ration was a commerical type layer-breeder that has been used in many experiments at Michigan State University. The formulation of the ration is shown in Table 2. The feed and water were available *ad libitum*.

At the beginning of the laying period, a 14 hour light period per day was established. A step-up lighting program (10 minutes per week) was used, with 17 hours of light per day being the maximum. Approximately, .65 foot candles of light were available to the birds at the feed trough level.

The laying period lasted for thirteen 28-day periods. All data, except feed consumption, were collected on a per cage basis. The feed consumption data were collected for a block of four cages of the same treatment. The feed was weighed at the end of each 28-day period. Data were recorded for egg production and mortality on a daily basis. All birds were necropsied as described previously. Egg weights, shell thickness, Haugh units and percent blood spots were recorded one day during the second and fourth week in each 28-day period. All eggs from each cage that were collected for the egg weights were weighed, but a maximum of four eggs were used for the shell thickness, Haugh units and percent blood spot data. The age at sexual maturity was based on 50 percent or greater egg production for three consecutive days. Body weights were taken on a random sample of 24 birds per treatment group at the end of the thirteenth 28-day period. At the end of the 13th laying period, 145 unvaccinated and 112 vaccinated birds were killed and a necropsy were performed on each bird by Dr. H. G. Purchase.

The egg production, egg weight, shell thickness, Haugh units, body weight, percent total mortality, percent blood spots, percent mortality caused by MD, percent mortality caused by lymphoid leukosis, and age at sexual maturity data were analyzed by the analysis of variance for a three way factorial plus replicate design. The percent total mortality, percent blood spot, percent mortality caused by MD and percent mortality caused by lymphoid leukosis data were transformed to arcsin percentage prior to performing the analysis of variance. Tables containing the analysis of variance figures will be presented only for the data where significant differences were found. The facilities at the computer laboratory were used for the data analysis.

The data for feed per dozen eggs were not statistically analyzed. The experimental design followed in the experiment was prepared by Gill (1969).

RESULTS AND DISCUSSION

Growing period

The total mortality, mortality caused by MD, feed consumption per bird, and 20 week body weight data are presented in Table 3. Total mortality for the unvaccinated birds was 23.8 percent as compared with a mortality of 8.6 percent for the vaccinated birds. The statistical analysis of the total mortality data indicated a highly significant ($P<0.01$) difference between the vaccinated and unvaccinated groups. Mortality caused by MD was seven and one-half times greater for the unvaccinated group than for the vaccinated group. The difference in MD mortality between the two groups was highly significant ($P<0.01$).

Table 3. Effect of HVT-vaccination on total mortality, Marek's disease mortality, feed consumed per bird and 20 week body weight.

Treatment	Total Mortality (Percent)	Marek's disease Mortality (Percent)	Feed Per bird (Kgs./bird)	20 week body weight (grams)
HVT-vaccinated	8.6b[1]	2.3b[1]	7.91b[1]	1383a[1]
Unvaccinated	23.8a	17.4a	8.20a	1438a

[1]Means under a given subheading not having the same superscript are significantly different at the 0.01 probability level.

As indicated in Figure I, the mortality among the unvaccinated birds reached a peak during the 13 to 16 week period and declined thereafter. The first diagnosis of MD in the unvaccinated group occurred furing the sixth week. The highest mortality (approximately 2.9 percent per four-week period) among the vaccinated birds was during the one to four week and 17 to 20 week periods. Within, the one to four week period most of the mortality was during the first week. These deaths were thought to be due to trauma from the intra-abdominal administration of the vaccine. The first death caused by MD in the vaccinated group occurred during the tenth week. The greatest MD mortality (approximately 1.5 percent per four week period) in the vaccinated group was during the 17 to 20 week period. In addition to Marek's disease other causes of death were lymphoid leukosis and non-specific causes which include cannibalism. Birds that were not necropsied because of post morteum decomposition were included in the non-specific causes.

Siccardi and Burmester (1970) mentioned that lymphoid leukosis usually appears when the birds are 16 weeks of age or older. The first diagnosis of lymphoid leukosis was during the seventeenth week for the vaccinated and unvaccinated birds.

In this experiment, the ability of the HVT vaccine to offer protection against MD in the growing pullet, agrees very strongly with the results presented by Purchase et al. (1971) and Edison et al. (1971).

It was noticed that in several instances where two cages contained unvaccinated birds were adjacent, most of the pullets in one cage had died of MD, whereas birds in the other cage were not affected

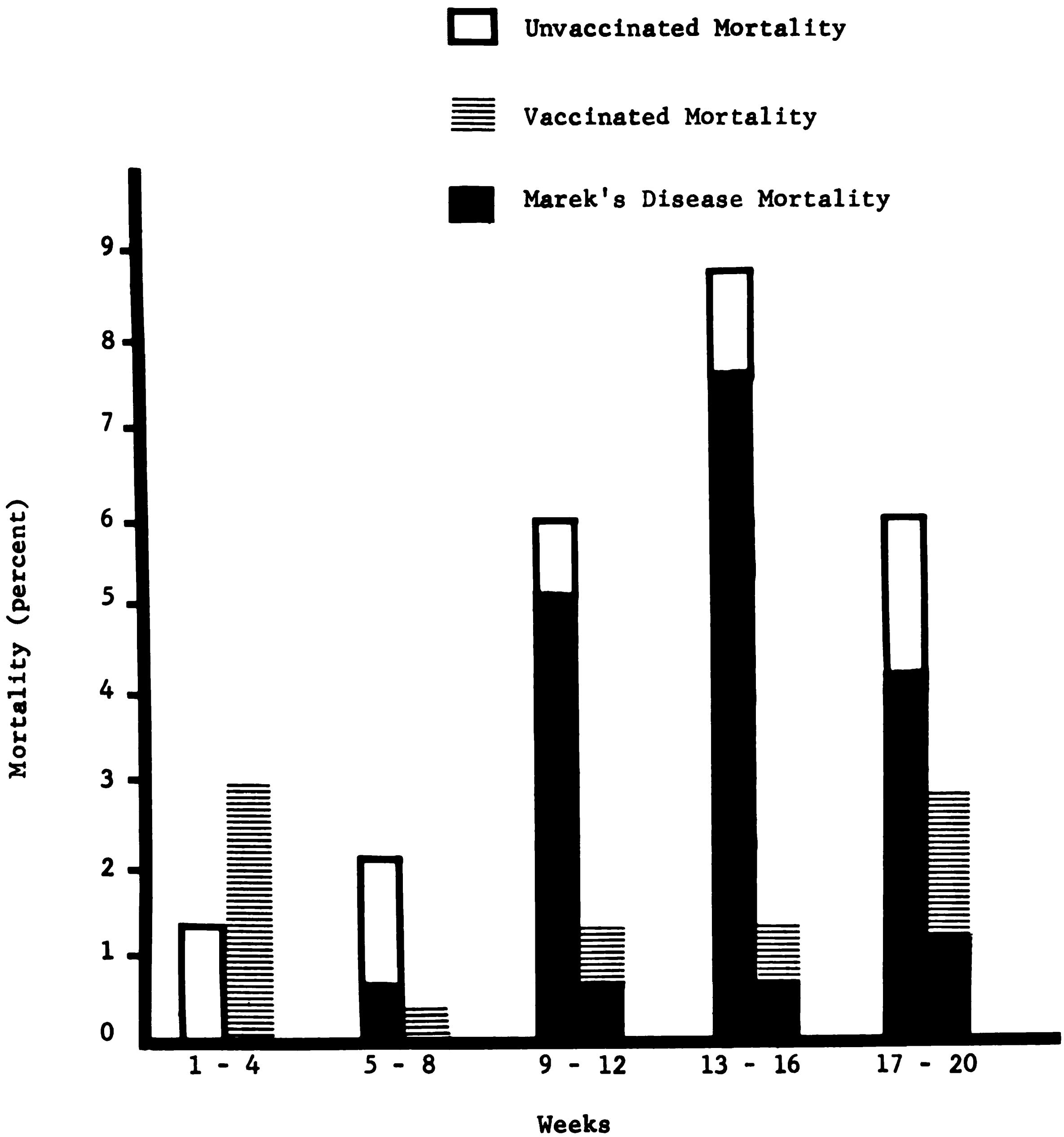

Figure 1. Percent total mortality and Marek's disease mortality for vaccinated and unvaccinated pullets for a 20 week growing period.

by MD. There seems to be no logical explanation.

The average 20 week body weight of the unvaccinated birds was 55 grams greater than the body weight of the vaccinated birds. This difference was not significant. Purchase et al. (1971) reported that HVT vaccination had no significant effect on body weight. In that study the vaccinated birds were slightly heavier than the unvaccinated birds.

The unvaccinated pullets consumed significantly ($P<0.01$) more feed per bird than did the vaccinated pullets. The feed consumed per bird for the unvaccinated group was 8.20 kgs. as compared with 7.91 kgs. per bird for the vaccinated group. The difference may have been affected by the high mortality of the unvaccinated birds. The mortality had the effect of increasing the floor space and feeder space available for each bird. At 20 weeks, the number of unvaccinated birds per cage ranged from three to eleven, while the number of vaccinated birds per cage ranged from eight to twelve. Wildey (1969) using the same cage and equipment reported in this study, conducted a cage density experiment with growing pullets. When the pullets reached 20 weeks of age, Wildey found that pullets at a density of five per cage consumed more feed per bird than did pullets at a density of 10 per cage. He stated that floor space and feeder space had an influence on the amount of feed consumed per bird.

There were 19 and 35 cull birds removed from the vaccinated and unvaccinated groups, respectively, when the birds reached 20 weeks of age. These birds were necropsied and 23 of the unvaccinated culls had MD lesions, while only one of the vaccinated culls had MD lesions.

At 20 weeks there were 611 vaccinated and 483 unvaccinated pullets that were healthy enough to be classified as saleable pullets. The estimated cost per saleable pullet is shown in Table 4. All costs were identical for both groups, except the feed consumed by each group and the cost of the HVT vaccine for the vaccinated group. The total cost of the vaccinated birds was $990.00 as compared with $923.00 for the unvaccinated birds. The cost per saleable vaccinated pullet was $1.62 and the cost per saleable unvaccinated pullet was $1.93. The high mortality in the unvaccinated group greatly affected that cost.

Table 4. Estimated cost per saleable 20 week old pullet.

Item	HVT-vaccinated	Unvaccinated
Chicks	$ 245	$ 245
House & Equipment	91	91
Feed	430	407
Heat	21	21
Debeaking	21	21
Vaccination	28	28
Miscellaneous	35	35
Labor	94	84
HVT vaccine	35	--
Total Cost	$ 990	$ 932
Number of saleable pullets	611	483
Cost per saleable pullet	$1.62	$ 1.93

Based on the data presented, it appears that vaccination with HVT is an effective means of protection against MD. Birds that are vaccinated with HVT should experience fewer deaths from MD than birds that had not been vaccinated against MD. The lower mortality allows the cost to grow a pullet to be greatly reduced and the $ .31 saving in growing cost per bird is a substantial amount.

Laying period

Egg production.-- The means for hen housed egg production are presented in Table 5. The analysis of variance for these data is shown in Table 6. The average means for birds on different treatments were: vaccinated 47.68 %, unvaccinated 39.28%, transported 44.15%, not transported 42.32%, eight birds/cage 44.55%, and 10 birds/cage 42.42%. Vaccination was found to have a highly significant ($P<0.01$) effect on hen housed egg production. This difference could be expected since the mortality in the unvaccinated group was much greater than mortality in the vaccinated group. The difference in mortality will be discussed later. Transporting of the birds and cage density had no significant effect on hen housed egg production. Also there were no significant interactions.

The means for hen day egg production are shown in Table 7. The average means for birds on different treatments were: vaccinated 59.01%, unvaccinated 58.27%, transported 58.20%, not transported 59.09%, eight birds/cage 59.75%, and 10 birds/cage 57.54%. An analysis of the egg production data on a hen day basis, shown in Table 8, revealed that density had a significant ($P<0.05$) effect on egg production. The difference in the means for density is in agreement with Ostrander and Young (1969), Fowler and Quisenberry

Table 5. Effect of HVT-vaccination, transporting and cage density on hen housed egg production.

	Hen housed egg production (percent)			
	8 birds/cage		10 birds/cage	
	Transported	Not Transported	Transported	Not Transported
HVT-vaccinated	50.60	46.99	46.74	46.42
Unvaccinated	42.31	38.31	36.96	39.57

Averages:	HVT-vaccinated	47.68	Unvaccinated	39.28
	Transported	44.15	Not transported	42.82
	8 birds/cage	44.55	10 birds/cage	42.42

Table 6. Analysis of variance for hen housed egg production.

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
HVT-vaccination	1	1693.18	1693.18	22.970**
Transporting	1	42.30	42.30	0.573
V x T	1	9.60	9.60	0.130
Density	1	109.16	109.16	1.480
V x D	1	0.17	0.17	.002
T x D	1	147.13	147.13	1.996
V x T x D	1	16.49	16.49	0.223
Error	88	6486.53	73.71	
Total	95	8504.56		

** Significant at the 0.01 level of probability.

Table 7. Effect of HVT-vaccination, transporting and cage density on hen day egg production.

	Hen day egg production (percent)			
	8 birds/cage		10 birds/cage	
	Transported	Not Transported	Transported	Not Transported
HVT-vaccinated	60.95	59.73	55.99	59.38
Unvaccinated	59.56	58.75	56.29	58.38

Averages:	HVT-vaccinated	59.01	Unvaccinated	58.27
	Transported	58.20	Not transported	59.09
	8 birds/cage	59.75	10 birds/cage	57.54

Table 8. Analysis of variance for hen day egg production.

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
HVT-vaccination	1	12.87	12.87	0.489
Transporting	1	19.18	19.18	0.714
V x T	1	0.92	0.92	0.034
Density	1	116.93	116.93	4.353*
V x D	1	4.82	4.82	0.179
T x D	1	87.27	87.27	3.249
V x T x D	1	3.76	3.76	0.140
Error	88	2363.65	26.86	
Total	95	2609.40		

*Significant at the 0.05 level of probability.

(1970) and Dorminey and Arscott (1971) who reported that as the number of birds in a given cage size increased, percent egg production tended to decrease. It is interesting to note that, on a hen day basis, vaccination did not have any effect on egg production.

The egg production percentages in this experiment are rather low. In addition to mortality, there may have been another factor which had an influence on egg production. The birds started into egg production during May and were in peak production during June, July, and August. During the three summer months, the temperature in the experimental room was often in the 85°F to 94°F range. Birds that are in a rather warm enviroment tend to not consume a sufficient quantity of feed to maintain a normal level of egg production.

Mortality.-- The means for the total mortality data are presented in Table 9. The analysis of variance for the data is shown in Table 10. It indicated that vaccination had a highly significant ($P<0.01$) effect on mortality. Neither transporting, density or any of the interactions had a significant effect on mortality. The average means for birds on different treatments were: vaccinated 27.97%, unvaccinated 43.59%, transported 34.64%, not transported 36.93%, eight birds/cage 34.90%, and 10 birds/cage 36.67%. As mentioned previously, the 15.62 percent difference in mortality between the vaccinated and unvaccinated groups caused the difference that existed in hen housed production for the two groups.

The means for MD mortality are shown on Table 11. The average means for birds on different treatments were: vaccinated 2.81%, unvaccinated 11.20%, transported 7.24%, not transported 6.77%, eight birds/cage 7.55%, and 10 birds/cage 6.46%. The analysis of variance of these data is presented in Table 12. There is a highly significant

Table 9. Effect of HVT-vaccination, transporting and cage density on total mortality.

	Total mortality (percent)			
	8 birds/cage		10 birds/cage	
	Transported	Not Transported	Transported	Not Transported
HVT-vaccinated	25.00	30.21	25.83	30.83
Unvaccinated	38.54	45.83	49.17	40.83

Averages:	HVT-vaccinated	27.97	Unvaccinated	43.59
	Transported	34.64	Not transported	36.93
	8 birds/cage	34.90	10 birds/cage	36.67

Table 10. Analysis of varience for total mortality (arcsin transformation).

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
HVT-vaccination	1	2374.57	2374.57	19.897**
Transporting	1	130.22	130.22	1.091
V x T	1	117.28	117.28	0.983
Density	1	42.20	42.20	0.354
V x D	1	15.82	15.82	0.133
T x D	1	199.84	199.84	1.167
V x T x D	1	167.56	167.56	1.404
Error	88	10502.13	119.34	
Total	95	13549.62		

** Significant at the 0.01 level of probability.

Table 11. Effect of HVT-vaccination, transporting and cage density on Marek's disease mortality.

	Marek's disease mortality (percent)			
	8 birds/cage		10 birds/cage	
	Transported	Not Transported	Transported	Not Transported
HVT-vaccinated	1.04	5.21	3.33	1.67
Unvaccinated	10.42	13.54	14.17	6.67

Averages:	HVT-vaccinated	2.81	Unvaccinated	11.20
	Transported	7.24	Not transported	6.77
	8 birds/cage	7.55	10 birds/cage	6.46

Table 12. Analysis of variance for Marek's disease mortality (arcsin transformation)

Source of variance	Degrees of freedom	Sum of square	Mean Square	F ratio
HVT-vaccination	1	3002.61	3002.61	26.984**
Transported	1	9.56	9.56	0.086
V x T	1	102.78	102.78	0.924
Density	11	0.76	0.76	0.007
V x D	1	0.19	0.19	0.002
T x D	1	643.82	643.82	5.786*
V x T x D	1	10.70	10.70	0.096
Error	88	9792.04	9792.04	
Total	95	13562.46	13562.46	

* Significant at the 0.05 level of probability.
** Significant at the 0.01 level of probability.

(P<0.01) difference between the vaccinated and unvaccinated means. Transporting and cage density had no significant effect on MD mortality. However, there is a significant (P<0.05) transporting X density (T x D) interaction. This indicates that MD mortality in the transported and not transported groups did not occur in a similar manner with respect to cage density. For the transported group, the greatest MD mortality occurred with 10 birds/cage, while in the not transported group, the greatest MD mortality, occurred with eight birds/cage. There seems to be no logical explanation why there should be a significant transporting x density interaction.

The means for lymphoid leukosis mortality are presented in Table 13. The average means for birds on different treatments were: vaccinated 13.54%, unvaccinated 22.81%, transported 18.28%, not transported 18.07%, eight birds/cage 18.23% and 10 birds/cage 18.13%. The analysis of variance in Table 14 showed the 9.27 percent difference in lymphoid leukosis mortality between the vaccinated and unvaccinated groups to be highly significant (P<0.01). The significant (P<0.05) vaccination x density (V x D) interaction implies that the occurence of lymphoid leukosis in the vaccinated and unvaccinated birds did not react the same with respect to cage density. The highest lymphoid leukosis mortality in the vaccinated group occurred with eight birds/cage, whereas the highest lymphoid leukosis mortality in the unvaccinated group occurred with 10 birds/cage. There seems to be no logical explanation why there should be a vaccination x density interaction. Also, there is no easy explanation forthcoming as to the reason the HVT vaccine had an effect on reducing lymphoid leukosis mortality.

Table 13. Effect of HVT-vaccination, transporting and cage density lymphoid leukosis mortality.

	Lymphoid leukosis mortality (percent)			
	8 birds/cage		10 birds/cage	
	Transported	Not Transported	Transported	Not Transported
HVT-vaccinated	17.71	15.63	7.50	13.33
Unvaccinated	18.75	20.83	29.17	22.50

Averages:	HVT-vaccinated	13.54	Unvaccinated	22.81
	Transported	18.28	Not transported	18.07
	8 birds/cage	18.23	10 birds/cage	18.13

Table 14. Analysis of variance for lymphoid leukosis mortality (arcsin transformation).

Source of variance	Degrees of freedom	Sum of squares	Mean of square	F ratio
HVT-vaccination	1	1843.63	1843.63	10.652**
Transported	1	22.87	22.87	0.132
V x T	1	146.87	146.87	0.849
Density	1	41.48	41.48	0.240
V x D	1	890.97	890.97	5.148*
T x D	1	0.97	0.97	0.006
V x T x D	1	280.78	280.78	1.622
Error	88	15231.13	173.08	
Total	95	18458.70		

* Significant at the 0.05 level of probability.
** Significant at the 0.01 level of probability.

As shown in Figure 2, the total mortality for the unvaccinated birds during the period one, was 6.9 percent. This compares with a total mortality of 3.5 percent for the vaccinated birds for the same period. Total mortality for the unvaccinated group reached a peak during period two, whereas total mortality for the vaccinated group peaked during period three. Except for period six, total mortality in the unvaccinated birds always exceeded that of the vaccinated birds. The greatest cause of mortality in vaccinated and unvaccinated groups for periods one, two , and three was lymphoid leukosis. The incidence of lymphoid leukosis for both groups reached a peak during period two and declined thereafter. As indicated, MD caused some deaths throughout the entire 13 production periods. In addition to Marek's disease and lymphoid leukosis other causes of death were leiomyoma, hemangioma and non-specific causes which include prolopse, and cannibalism. The birds that were not necropsied because of post morteum decomposition were listed in the non-specific causes of death.

The data presented for mortality have shown that the HVT vaccine provided protection for laying hens against MD. At this time a valid explanation cannot be offered as to the reason why the vaccinated birds had a lower lymphoid leukosis mortality than did the unvaccinated birds.

Age at sexual maturity.-- The means for age at sexual maturity are presented in Table 15. The average means for birds on different treatments were: vaccinated 177.1 days, unvaccinated 177.2 days, transported 178.4 days, not transported 175.9 days, eight birds/cage 177.1 days, and 10 birds/cage 177.2 days. The age at sexual

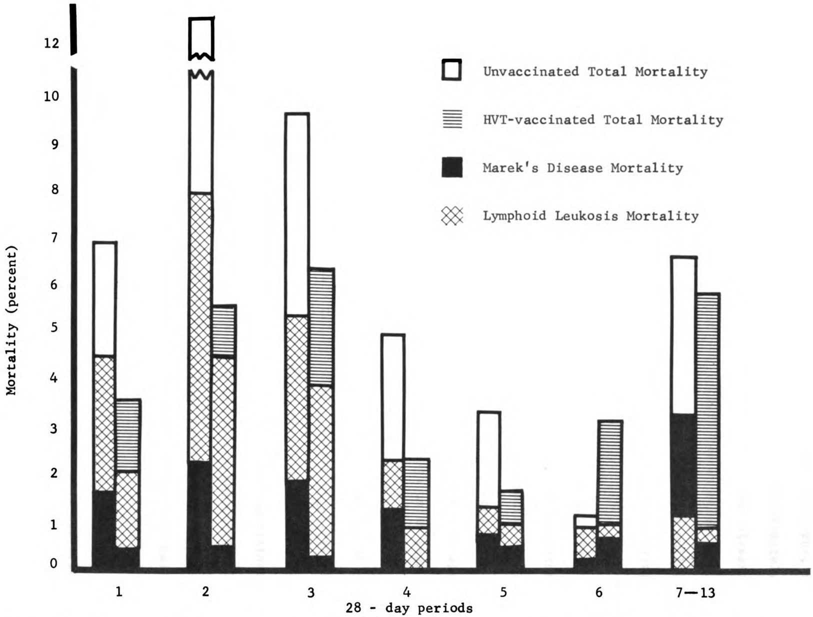

Figure 2. Percent total mortality, Marek's disease mortality and lymphoid leukosis mortality for HVT-vaccinated and unvaccinated pullets for thirteen 28-day periods.

maturity was significantly ($P<0.05$) affected by transporting as indicated by the analysis of variance in Table 16. The difference in the transported and not transported average means is approximately three days. A possible explanation would be that the many stresses associated with the transporting of the pullets had an effect of weakening their physical condition. If this were true, the pullets would need two or three days to readjust to conditions in a laying cage.

Haugh units.-- The means for the Haugh unit scores are presented in Table 17. The average means for eggs from the different treatments were: vaccinated 76.90 Haugh units, unvaccinated 76.38 Haugh units, transported 76.19 Haugh units, not transported 77.08 Haugh units, eight birds/cage 76.54 Haugh units, and 10 birds/cage 76.73 Haugh units. The analysis of variance for Haugh units (egg quality) in Table 18, indicated that transporting has a significant ($P<0.05$) effect on egg quality. A Haugh unit score for 76 or 77 indicates a U.S.D.A. quality rating of AA. Welter et al. (1967) reported that transporting of pullets had an effect of reducing egg quality. Their birds were transported (under simulated conditions) at 20 weeks of age at a temperature of 38°F. Welter and co-workers suggested that the difference in Haugh unit values was a result of possible damage to the oviduct during the time the birds were being cooped and transported.

Blood spots.--The means for percent blood spots are shown in Table 19. The average means for blood spots in eggs from the different treatments were: vaccinated 5.33%, unvaccinated 5.83%, transported 5.78%, not transported 5.37%, eight birds/cage 4.96%, and

Table 15. Effect of HVT-vaccination, transporting and cage density on the age at sexual maturity.

	Age at sexual maturity (days)			
	8 birds/cage		10 birds/cage	
	Transported	Not Transported	Transported	Not Transported
HVT-vaccinated	177.3	176.8	180.0	174.0
Unvaccinated	177.5	176.8	178.5	176.0

Averages:	HVT-vaccinated	177.1	Unvaccinated	177.2
	Transported	178.4	Not transported	175.9
	8 birds/cage	177.1	10 birds/cage	177.2

Table 16. Analysis of variance for age at sexual maturity.

Source of variance	Degrees of freedom	Sum of squares	Mean of square	F ratio
HVT-vaccination	1	0.38	0.38	0.010
Transported	1	145.04	145.04	3.992*
V x T	1	16.67	16.67	0.459
Density	1	0.04	0.04	0.001
V x D	1	0.17	0.17	0.005
T x D	1	80.67	80.67	2.220
V x T x D	1	22.04	22.04	0.607
Error	88	3197.50	36.34	
Total	95	3462.51		

* Significant at the 0.05 level of probability.

Table 17. Effect of HVT-vaccination, transporting and cage density on Haugh units.

	Haugh units			
	8 birds/cage		10 birds/cage	
	Transported	Not Transported	Transported	Not Transported
HVT-vaccinated	75.75	78.25	76.75	76.83
Unvaccinated	75.75	76.42	76.50	76.83

Averages:	HVT-vaccinated	76.90	Unvaccinated	76.38
	Transported	76.19	Not transported	77.08
	8 birds/cage	76.54	10 birds/cage	76.73

Table 18. Analysis of variance for Haugh units.

Source of variance	Degrees of freedom	Sum of squares	Mean Square	F ratio
HVT-vaccination	1	6.51	6.51	1.290
Transported	1	19.26	19.26	3.815*
V x T	1	3.76	3.76	0.745
Density	1	0.84	0.84	0.167
V x D	1	3.76	3.76	0.745
T x D	1	11.34	11.34	2.247
V x T x D	1	6.51	6.51	1.290
Error	88	444.25	5.05	
Total	95	496.23		

* Significant at the 0.05 level of probability.

10 birds/cage 6.19%. A difference of 1.23 percent existed between the eight birds/cage and 10 birds/cage groups and this difference did approach significance ($P<0.06$). This possibily suggests that the more crowded conditions in the 10 birds/cage has an influence on the rupturing of blood vessels in the ovarian follicle during ovulation.

Egg weight.--The means for egg weight are presented in Table 20. the average means for egg weights from the different treatments were: vaccinated 60.14 grams, unvaccinated 59.59 grams, transported 59.67 grams, not transported 60.07 grams, eight birds/cage 60.04 grams, and 10 birds/cage 59.68 grams. No significant differences were found.

Shell thickness.--The means for shell thickness are shown in Table 21. The average mean for shell thickness of eggs from the different treatments were: vaccinated .347 millimeters, unvaccinated .347 millimeters, transported .348 millimeters, not transported .346 millimeters, eight birds/cage .346 millimeters, and 10 birds/cage .348 millimeters. No significant differences were found.

Body weight.--The means for body weight are shown in Table 22. The average means for birds on the different treatments were: vaccinated 2008 grams, unvaccinated 1976 grams, transported 2000 grams, not transported 1978 grams, eight birds/cage 2004 grams, and 10 birds/cage 1980 grams. None of the differences were found to be significant.

Feed per dozen eggs.--In Table 23, are presented the means for the kilograms of feed required per dozen eggs. The average means for feed per dozen eggs for the different treatments were as follows: vaccinated 2.26 Kgs./dozen, unvaccinated 22.25 Kgs./dozen, transported 2.25 Kgs./dozen, not transported 2.26 Kgs./dozen, eight birds/cage

Table 19. Effect of HVT-vaccination, transporting and cage density on blood spots in eggs.

	Blood spots (percent)			
	8 birds/cage		10 birds/cage	
	Transported	Not Transported	Transported	Not Transported
HVT-vaccinated	5.06	5.12	6.37	4.77
Unvaccinated	4.29	5.37	7.41	6.24

Averages:	HVT-vaccinated	5.33	Unvaccinated	5.83
	Transported	5.78	Not transported	5.37
	8 birds/cage	4.96	10 birds/cage	6.19

Table 20. Effect of HVT-vaccination, transporting and cage density on egg weight.

	Egg Weight (grams)			
	8 birds/cage		10 birds/cage	
	Transported	Not Transported	Transported	Not Transported
HVT-vaccinated	59.72	60.63	59.77	60.44
Unvaccinated	60.33	59.52	58.85	59.68

Averages:	HVT-vaccinated	60.14	Unvaccinated	59.59
	Transported	59.67	Not transported	60.07
	8 birds/cage	60.04	10 birds/cage	59.68

Table 21. Effect of HVT-vaccination, transporting and cage density on egg shell thickness.

	Egg shell thickness (millimeters)			
	8 birds/cage		10 birds/cage	
	Transported	Not Transported	Transported	Not Transported
HVT-vaccinated	.350	.342	.348	.350
Unvaccinated	.348	.344	.348	.348

Averages:	HVT-vaccinated	.347	Unvaccinated	.347
	Transported	.348	Not transported	.346
	8 birds/cage	.346	10 birds/cage	.348

Table 22. Effect of HVT-vaccination, transporting and cage density on body weight.

	Body weight (grams)			
	8 birds/cage		10 birds/cage	
	Transported	Not Transported	Transported	Not Transported
HVT-vaccinated	2053	1918	1979	1955
Unvaccinated	2057	1989	1936	2051

Averages:	HVT-vaccinated	2008	Unvaccinated	1976
	Transported	2000	Not transported	1978
	8 birds/cage	2004	10 birds/cage	1980

Table 23. Effect of HVT-vaccination, transporting and cage density on kilograms of feed per dozen eggs.

	Feed (Kgs.)/dozen eggs			
	8 birds/cage		10 birds/cage	
	Transported	Not Transported	Transported	Not Transported
HVT-vaccinated	2.16	2.31	2.31	2.24
Unvaccinated	2.24	2.33	2.27	2.17

Averages:	HVT-vaccinated	2.26	Unvaccinated	2.25
	Transported	2.25	Not transported	2.26
	8 birds/cage	2.26	10 birds/cage	2.25

2.26 Kgs./dozen, and 10 birds/cage 2.25 Kgs./dozen. These data were not analyzed statistically. However, it appears that no significant differences would exist.

Post laying period examination.--At the end of the laying phase of the experiment, 112 vaccinated and 145 unvaccinated birds were killed and necropsied. In the vaccinated group, four birds were found to have leiomyoma. None of the vaccinated birds had any MD lesions. In the unvaccinated group, 21 birds had leiomyoma and three had MD lesions. Leiomyoma, as defined by Stedman's medical dictionary, is a benign tumor consisting largely of smooth muscle cells. The tumors may be found in any position where there is pre-existing smooth muscle. Purchase (1971) commented that leiomyoma was a common condition in laying hens that had been in production for approximately one year.

Economic implications.--There are important economic implications that can be drawn from the egg production data. It appears that it would be to the advantage of the commercial egg producer to vaccinate his chicks with the HVT vaccine or to buy pullets that have been vaccinated with the HVT vaccine. The following practical application is based on the assumptions that (1) hen housed egg production would be approximately 63 percent for vaccinated birds and 58 percent for unvaccinated birds, (2) the cost to produce a dozen eggs for each group is equal, (3) egg size distribution is equal, (4) blend egg price is $.30 per dozen, and (5) laying flock size is 30,000 hens. Over a period of one year, the vaccinated birds would produce 574,833 dozen eggs, yielding a gross income of $172,450. The unvaccinated birds would produce 529,250 dozen eggs having a gross value of $158,775. A comparison of the gross incomes shows that the vaccinated birds would have an advantage of $13,675.

Although the transporting of 20 week old pullets had no significant effect on reducing egg production, the cost involved in transporting birds must be considered by the poultryman. The catching, cooping, loading, transporting, unloading and caging of pullets is a costly operation. One of Michigan's larger poultry operators (Ananymous, 1971) stated that it costs $.07 per bird to move a pullet from a growing house to a laying house. A breakdown of the cost is (1) catching and putting the birds in coops - one cent per bird, (2) loading, transporting, and unloading the coops - five cents per bird, and (3) uncooping and putting the birds into laying cages - one cent per bird. This cost of seven cents per bird to move them from a growing house to a laying house can be a substantial amount, if large numbers of birds are involved. There seems to be a dire need for a cash flow analysis which would compare a start-grow-lay cage operation with a typical cage layer operation.

A practical application can also be illustrated for the poultryman engaged in a pullet growing enterprize. The following example is based on the assumptions that: (1) mortality for a 20 week growing period would be approximately five percent for pullets vaccinated with HVT and 12 percent for unvaccinated pullets, (2) a total of 14,206 growing cages, and (3) an order of 100,000 20 week old pullets. To fill an order for 100,000 pullets, the grower would have to start approximately 113,650 one day old chicks if they had not been vaccinated with HVT. To start the chicks, the grower would need 14,206 growing cages. However, by vaccinating the chicks with HVT, the grower would only need to start approximately 105,300 chicks. To house the vaccinated chicks, 13,162 cages would be used and this means that 1,044 cages would not be needed. A problem arises if the grower vaccinated the chicks with HVT and continues to fully utilize his cages (113,650 birds) to fill the order for 100,000 pullets. He would then have 107,968 pullets available at 20 weeks to fill the order of 100,000 pullets. These "extra" 7,968 pullets would probably be sold and would eventually begin to produce eggs. The problem of extra pullets is greatly magnified if hundreds of growers in the United States continue to start a given number of chicks based on their past experiences with MD. These "extra" pullets could have an effect of creating a surplus condition in the egg market.

For example, in 1970 when none of the laying hens were vaccinated with HVT, there were approximately 316 million laying hens in the United States. These hens produced approximately 70 billion eggs, and 1970 was a year of relatively low egg income at the farm. Therefore, by vaccinating with HVT and filling the growing houses to capacity there could be approximately 340 million laying hens. This increase in laying hen numbers could be entirely due to the lower mortality of the vaccinated pullets. If 316 million laying hens created a low price condition in 1970, then 340 million laying hens would definitely create a surplus condition.

SUMMARY

Growing period

Seven hundred one-day-old egg type chicks were vaccinated with 9,800 plaque forming units of a herpesvirus of turkeys (HVT), strain FC 126, per chick. Another 700 chicks were not vaccinated. The chicks were housed in a start-grow-lay cage system with 25 chicks per cage. The vaccinated and unvaccinated chicks were not intermingled in the same cage. Data were collected for mortality, feed consumption and body weight for the 20 week growing period.

Total mortality for the unvaccinated pullets was over two and one-half times greater (23.8 percent vs. 8.6 percent) than total mortality for the vaccinated pullets. This difference was highly significant ($P<0.01$). Mortality caused by Marek's disease (MD) was seven and one-half times greater (17.4 percent vs. 2.3 percent) for the unvaccinated birds than for the vaccinated birds. This difference was highly significant ($P<0.01$). The first diagnosis of MD in the unvaccinated group occurred furing the sixth week as compared with the tenth week for the vaccinated birds.

The unvaccinated birds consumed significantly ($P<0.01$) more feed per bird than did the vaccinated birds. At 20 weeks of age there was no significant difference in body weights of the vaccinated and unvaccinated groups.

When the birds reached 20 weeks of age, 19 and 35 cull birds were removed from the vaccinated and unvaccinated groups, respectively. These birds were necropsied and 23 of the unvaccinated culls had MD lesions, while only one of the vaccinated culls had MD lesions.

At 20 weeks, there were 611 vaccinated and 483 unvaccinated pullets that were classified as saleable pullets. By applying commercial cost figures, the cost per saleable vaccinated pullets was $1.62 and the cost per saleable unvaccinated pullet was $1.93.

The data obtained during the growing period indicated that vaccination with HVT was a safe and effective method of protecting against MD. Birds that are vaccinated with HVT should experience fewer deaths caused by MD than birds that have not been vaccinated with HVT.

Laying period

A 3 x 2 factorial experimental design was used for the laying period. The three factors were HVT-vaccination, transporting and cage density. Thus, the birds could be in any treatment combination of vaccinated or unvaccinated, transported or not transported and eight birds/cage or 10 birds/cage. The eight treatment combinations were (1) vaccinated, transported, eight birds/cage; (2) vaccinated, transported, 10 birds/cage; (3) vaccinated, not transported, eight birds/cage; (4) vaccinated, not transported,10 birds/cage; (5) unvaccinated, transported, eight birds/cage; (6) unvaccinated, transported, 10 birds/cage; (7) unvaccinated, not transported, eight birds/cage; and (8) unvaccinated, not transported, 10 birds/cage. Each treatment was replicated 12 times. A total of 432 vaccinated and

432 unvaccinated birds from the growing period were used in the laying period. The cages and equipment used in the growing period were also used in the laying period.

The procedures for transporting birds were similar to those used by the commercial poultry industry. The birds were transported for a period of five hours and a distance of 175 miles. The average outside temperature was 40°F. At the end of the transporting period, the birds were returned to the start-grow-lay cages at a density of eight or 10 birds/cage, also, the birds that were not transported were placed at a density of eight or 10 birds/cage.

Data were collected during thirteen 28-day periods on the performance of the pullets in the various treatment groups.

Vaccination was found to have a highly significant ($P<0.01$) effect on hen housed egg production. The average mean for the vaccinated birds was 47.68 percent as compared with 39.28 percent for the unvaccinated birds. An analysis of the egg production data on a hen day basis revealed that density had a significant ($P<0.05$) effect on egg production. The average mean for eight birds/cage was 59.75 percent and 57.54 percent for 10 birds/cage.

Vaccination had a highly significant ($P<0.01$) effect on total mortality, MD mortality and lymphoid leukosis mortality. The average mean for total mortality in the vaccinated group was 27.97 percent and 43.59 percent for total mortality in the unvaccinated group. For MD mortality, the average mean for the vaccinated birds was 2.81 percent as compared with 11.20 percent for the unvaccinated birds. The average mean for lymphoid leukosis mortality in the vaccinated group was 13.54 percent and 22.81 percent in the unvaccinated group.

The age at sexual maturity and egg quality (Haugh units) were significantly ($P<0.05$) affected by transporting of the pullets. The egg weights, percent blood spots, egg shell thickness, and body weights were not significantly affected by any of the factors. There were no differences among the treatment groups for the feed required to produce a dozen eggs.

The data obtained leads to the conclusion that the HVT vaccine offers protection against MD during the laying period. There also appears to be some beneficial effect resulting in a lower incidence of lymphoid leukosis mortality for birds vaccinated with HVT. When applying the mortality data to a commercial situation, the egg producer should also expect a greater hen housed egg production from birds vaccinated with the HVT vaccine.

REFERENCES

A.A.A.P. Report, 1967. Methods in Marek's disease research. Avian Diseases, 14:820-828.

Adams, A. W., and M. E. Jackson, 1968. Effect of cage size and bird density on performance of six commerical strains of layers. Poultry Sci. 47:1651.

Andrewes, C. H., and R. E. Glover, 1957. A case of neurolymphomatosis in a turkey. Vet. Rec. 51:934-935.

Anonymous, 1971. Personal Communication.

Beasley, J. N., L. T. Patterson and D. H. McWade, 1970. Transmission of Marek's Disease by poultry house dust and chicken dander. Amer. J. Vet. Res. 31:339-344.

Bell, D. D., and T. M. Little, 1966. Cage density as it relates to debeaking methods. Poultry Sci. 45:1069.

Biggs, P. M., and L. N. Payne, 1963. Transmission experiments with Marek's disease (fowl paralysis). Vet Rec. 75:177-179.

Biggs, P. M., and L. N. Payne, 1964. Relationship of Marek's disease (neural lymphomatosis) to lymphoid leukosis. N. C. I. Monograph No. 17, p. 83-98.

Biggs, P. N., L. N. Payne, B. S. Milne, A. E. Churchill, R. C. Chubb, D. G. Powell and A. H. Harris, 1970. Field trials with an attenuated cell associated vaccine for Marek's disease. Vet. Rec. 87:704-709.

Calnek, B. W., and S. B. Hitchner, 1969. Localization of viral antigen in chickens infected with Marek's disease herpesvirus. J. Natl. Cancer Inst. 43:935-949.

Calnek, B. W. and R. L. Witter, 1971. Marek's disease subchapter. Diseases of Poultry, 6th ed. Iowa State University Press (in press).

Calnek, B. W., H. K. Adldinger and D. E. Kahn, 1970. Feather follicle epithelium: a source of enveloped and infectious cell-free herpesvirus from Marek's disease. Avian Diseases, 14:219-233.

Champion, L. R., 1971. Personal communication.

Champion, L. R., and H. C. Zindel, 1968. Performance of layers in single and multiple bird cages. Poultry Sci. 47:1130-1135.

Champion, L. R., H. C. Zindel, R. K. Ringer and J. H. Wolford, 1966. The performance of started pullets treated with Su-9064 (Pacitran) prior to transport. Poultry Sci. 45:1359-1368.

Churchill, A. E., L. N. Payne, and R. C. Chubb, 1969. Immunization against Marek's disease using a live attenuated virus. Nature, 221:744-747.

Dorminey, R. W., and G. H. Arscott, 1971. Effects of bird density, nutrient density and perches on the performance of caged white Leghorn Layers. Poultry Sci. 50:619-626.

Eidson, C. S., and D. P. Anderson, 1970. Immunization against Marek's disease. Avian Diseases, 14:68-81.

Edison, C. S., D. P. Anderson, S. H. Kleven and J. Brown, 1970. Field trials of vaccines for Marek's disease. Avian Diseases, 14:312-322.

Fowler, J. C., and J. H. Quisenberry, 1970. Influence of pullet body weight and cage size on laying performance. Poultry Sci. 49:1385.

Gill, J. L., 1969. Personal communication.

Jungher, E., 1939. Neurolymphomatosis phasianorum. J. Amer. Vet. Med. Assoc. 94:49-52.

Kawamura, H., D. J. King and D. P. Anderson, 1969. A herpesvirus isolated from kidney cell culture of normal turkeys. Avian Diseases, 13:853-863.

Lowe, R. W., and B. W. Heywang, 1964. Performance of single and multiple caged White Leghorn layers. Poultry Sci. 43:801.

Marek, J., 1907. Multiple neruenentzundung bei huhnern. Dt. Tierarztl. Wschr. 15:417-421.

Marks, H. L., L. D. Tindell and R. H. Lowe, 1970. Performance of egg production stocks under three cage densities. Poultry Sci. 49:1094-1100.

Marr, J. E., and D. E. Green, 1970. Cage size and social density for laying hens. Poultry Sci. 49:1410-11.

Marr, J. E., D. W. Cardin, D. E. Green and J. L. Williamson, 1967. Evaluation of cage density for laying hens. Poultry Sci. 46:1289.

Mather, F. B., and E. W. Gleaves, 1970. Performance of commerical stocks of layers as influenced by cage density. Poultry Sci. 49:1412.

Moore, B. W., R. Plumley and H. M. Hyre, 1965. A cage density study of laying hens. Poultry Sci. 44:1339.

Nazerian, K., J. J. Solomon, R. L. Witter and B. R. Burmester, 1968. Studies on etiology of Marek's disease II. Finding of a herpesvirus in cell culture. Proc. Sci. Exp. Biol. Med. 127:177-182.

Okazaki, W., H. G. Purchase and B. R. Burmester, 1970. Protection against Marek's disease by vaccination with a herpesvirus of turkeys. Avian Diseases, 14:413-429.

Ostrander, C. E., and R. J. Young, 1969. Effect of density in cages on egg production, feed efficiency, egg size and economies. Poultry Sci. 48:1855.

Owings, W. J., S. L. Balloun, W. W. Marion and J. M. J. Ning, 1967. The influence of dietary protein level and bird density in cages on egg production and liver fatty acids. Poultry Sci. 46:1303.

Parker, J. E., and J. B. Rogers, 1954. Open houses and cages for laying hens. Oregon Agr. Expt. Sta. Bul. 543.

Purchase, H. G., 1971. Personal communication.

Purchase, H. G., W. Okazaki and B. R. Burmester, 1971. Field trials with the herpesvirus of turkeys (HVT) strain FC-126 as a vaccine against Marek's disease. Poultry sci. 50:775-783.

Quisenberry, J. H., and J. W. Bradley, 1964. Effects of bird density on performance of egg production stock. Poultry Sci. 43:1354.

Ruggles, L. H., D. L. Anderson, R. A. Damon and R. M. Grover, 1967. The effects of bird density, light intensity and diet on the performance of heavy type layers in cages. Poultry Sci. 46:1313.

Schupe, W. D., and J. H. Quisenberry, 1961. Effect of certain rearing and laying house environments on performance of incross egg production type pullets. Poultry Sci. 40:1165-1171.

Siccardi, F. J., and B. R. Burmester, 1970. The differential diagnosis of lymphoid leukosis and Marek's disease. U.S.D.A. Tech. Bull. No. 1412.

Snedecor, G. W., 1956. Statistical Methods, 5th Ed., Iowa State College Press, Ames, Iowa.

Solomon, J. J., R. L. Witter, K. Nazerian and B. R. Burmester, 1968. Studies on the etiology of Marek's disease I. Propagation of the agent in cell culture. Proc. Soc. Exp. Biol. Med. 127:173-177.

Tower, B. A., A. J. Olinde, F. R. Baker and E. P. Roy, 1967. Performance of layers confined in single versus colony cages. Poultry Sci. 46:1330.

Wayman, O., T. Pirzada, R. B. Herrick and K. Morita, 1969. A further study on the effect of concentration of the performance of caged layers in Hawaii. Hawaii Agr. Expt. Sta. Res. Rept. 173.

Welter, J. R., M. A. Boone and B. D. Barnett, 1967. The effects of temperature stress on subsequent egg production of the fowl. Poultry Sci. 46:646-650.

Weston, C. R., and J. H. Smith, 1969. Vaccination of day-old chicks for Marek's disease. Poultry Sci. 48:1891.

Wight, P. A. L., 1963. Lymphoid leukosis and fowl paralysis in the quail. Vet. Rec. 75:685-687.

Wildey, H. E., 1969. The laying house performance of chickens brooded and reared on floors and in cages. Doctorial Thesis, Michigan State University.

Wilson, H. R., J. E. Jones and R. W. Dorminey, 1967. Performance of layers under various cage regimes. Poultry Sci. 46:422-425.

Witter, R. L., B. W. Calnek and P. O. Levine, 1966. Occurrence of lymphomatosis in chickens free of resistance-inducing factor (RIF) virus. Avian Diseases, 12:522-530.

Witter, R. L., G. H. Burgoyne and J. J. Solomon, 1969. Evidence for a herpesvirus as an etiologic agent of Marek's disease. Avian Diseases, 13:171-184.

Witter, R. L., K. Nazerian, H. G. Purchase and G. H. Burgoyne, 1970. Isolation from turkeys of a cell-associated herpesvirus antigenically related to Marek's disease virus. Amer. J. Vet. Res. 31:525-538.