THE EFFECT OF RAW SOYBEAN DIETS AND CERTAIN SANITARY MEASURES UPON DISEASE INCIDENCE AND NUTRITIONAL PERFORMANCE OF CHICKENS

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Harbhajan Singh

1964

This is to certify that the

thesis entitled

THE EFFECT OF RAW SOYBEAN DIETS AND CERTAIN SANITARY MEASURES UPON DISEASE INCIDENCE AND NUTRITIONAL PERFORMANCE OF CHICKENS

presented by

HARBHAJAN SINGH

has been accepted towards fulfillment
of the requirements for

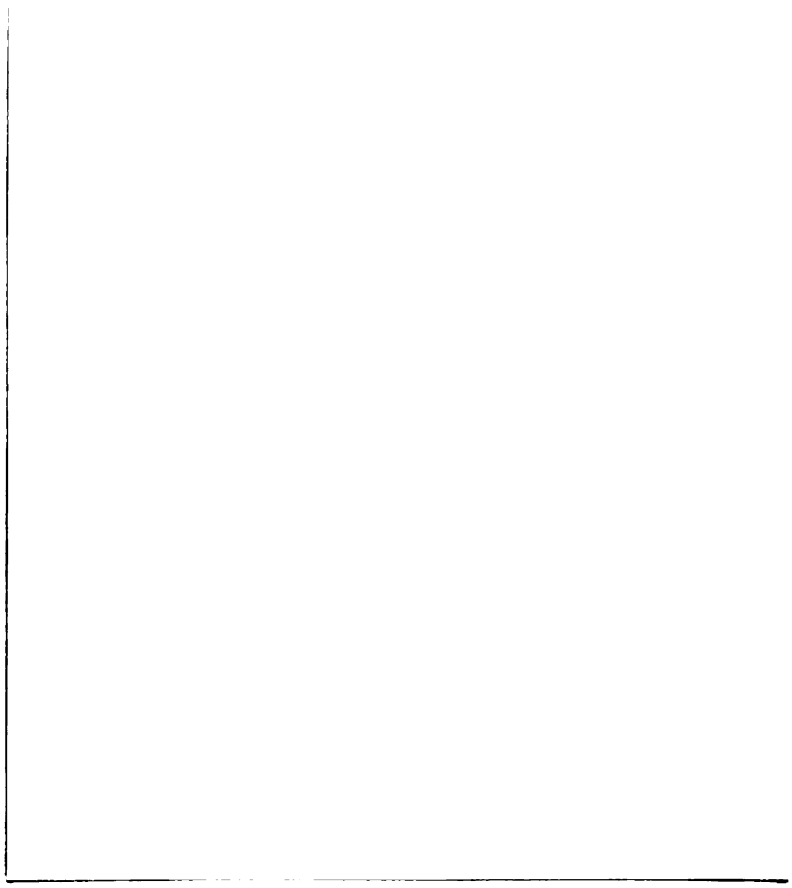
Ph.D. degree in Poultry Science

Philip J. Schaible
Major professor

Date May 18, 1964

O-169

ROOM USE ONLY



ABSTRACT

THE EFFECT OF RAW SOYBEAN DIETS AND CERTAIN SANITARY MEASURES UPON DISEASE INCIDENCE AND NUTRITIONAL PERFORMANCE OF CHICKENS

by Harbhajan Singh

Eight preliminary experiments were conducted using chickens of different breeds and varieties to determine the effects of chlorination of water on its consumption, weight of birds and egg production. Experiments were than undertaken with commercially-hatched White Leghorn-type hens to study the effects of certain sanitary management practices as well as dietary treatments on egg production and rate of mortality. The sanitary measures applied were: chlorinated drinking water and/or frequent litter change. Disinfectant pads were placed at the entrance to each experimental pen. Dietary treatments consisted of the use of soybean meal, whole raw soybeans, whole cooked soybeans, or the combination of raw and cooked soybeans in isocaloric and isonitrogenous rations. The management measures were followed in an attempt to see if experimental birds kept in a cleaner environment would be healthier and produce better.

Birds dying during the experiment were taken promptly to the laboratory for necropsy in order to investigate the cause of death. At the end of the experiment, blood was collected for the following laboratory tests: differential leukocytic counts, coagulation time, urea nitrogen and sugar.

The criteria used for evaluation were rate of mortality, relative infectiveness of the litter to baby chicks, rate of egg production, gain or loss in body weights of birds, egg size, fertility and hatchability of eggs from the hens receiving various dietary and management treatments.

In the preliminary experiments, the following levels of chlorine in water did not affect the feed or water consumption.

50 ppm for 3-week-old chicks

75 ppm for 10-week-old chicks

25 ppm for laying hens

Chlorination of the water at 100 to 1,000 ppm for 5-week-old chickens or 50 to 500 ppm for old hens lowered water intake but did not affect feed consumption or body weights.

Water and feed consumption, as well as gain in body weight were depressed by chlorination of water at levels above:

100 ppm for baby chicks

1,000 ppm for 5-week-old chickens

500 ppm for laying hens

Chlorination at levels of 50 ppm depressed egg production in laying hens.

Analysis of the data showed no significant differences among the various management practices as far as the mean egg production or the mortality rates were concerned. A significant difference ($P < .01$), however, occurred in egg production from different dietary treatments.

The soybean oil meal containing ration produced the best results but the rate of production of birds on this ration was not significantly better than that of birds on the experimental diet containing raw soybeans with supplementation of methionine and antibiotic, Pro-strep.

There was less mortality among birds fed soybean meal containing diets than among those fed raw soybeans with supplemental methionine, Pro-strep with or without detergent; however, this difference was not significant. Cannibalism, leukosis, ruptured yolks and peritonitis were the primary causes of death. Other pathological conditions diagnosed were sinusitis, nephritis,

hepatitis, lymphoid mesenteric tumor, synovitis and impacted oviduct. Infections associated with reproductive systems appeared to be more common than others.

Differential leukocytic counts, coagulation time, plasma proteins and blood urea nitrogen showed little variation among birds of different groups. Blood sugar levels were significantly ($P < .01$) higher in birds given both chlorinated water and litter change treatments than in birds given tap water with litter change treatments.

Various dietary and management treatments had similar effect on average egg weight, Haugh scores and egg shell thickness. Improvement in hatchability of eggs produced by birds kept on built-up litter was not evident.

Baby chicks brooded on litter previously used by hens kept under different management treatments did not differentiate between relative infectiveness of different litter systems.

There were no significant differences in bacterial counts in samples examined from different litter treatments. During winter the moisture content of the litter was higher in the treatments receiving frequent litter change than that of built-up litter. Different litter treatments had similar effects on coccidial oocysts.

The results of these studies suggest that chlorination of drinking water and better housekeeping such as more frequent litter changes or both may not be advantageous over the usual tap water and "built-up" litter practices, when judged on basis of mortality. This conclusion may not be applicable during an outbreak of disease when better housekeeping may be a primary step towards the control of such an outbreak.

THE EFFECT OF RAW SOYBEAN DIETS AND CERTAIN SANITARY MEASURES UPON DISEASE INCIDENCE AND NUTRITIONAL PERFORMANCE OF CHICKENS

by

Harbhajan Singh

A THESIS

SUBMITTED TO

Michigan State University

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Department of Poultry Science

1964

ACKNOWLEDGEMENTS

To: Dr. Philip J. Schaible for the guidance and advice that made this work possible.

Dr. T. H. Coleman, whose suggestions were most helpful and whose association will be a pleasant experience of my stay at Michigan State University.

Dr. Howard C. Zindel, Chairman of Department of Poultry Science and Professor J. A. Davidson for making this work possible in the form of a research assistantship.

Michigan State University, Department of Poultry Science and the people of Michigan for the use of farm facilities and poultry science laboratories.

Drs. H. C. Zindel, T. H. Coleman, C. C. Ellis and W. W. Snyder for constructive review of this manuscript.

Drs. C. C. Ellis, D. A. Schmidt, Dept. of Veterinary Pathology, Dr. L. G. Harmon, Department of Food Science and Dr. W. L. Mallmann, Department of Microbiology and Public Health for their assistance in this study.

My wife, Sharanjet, for her constant interest and encouragement throughout the course of study for this degree.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

INTRODUCTION AND REVIEW OF LITERATURE

Domestic fowl are susceptible to various ailments that impair their health and interfere with normal growth or egg production. The loss of chicken meat and eggs due to disease, parasitism and nutritional deficiencies amounts to approximately one-quarter billion dollars a year in the United States. This loss includes not only the market fowl value but lowered egg production, wasted feed and labor as well as reduced returns on the overall investment. In 1962, the loss from laying flocks alone was estimated to be 80 million dollars (Snyder et al., 1962). From 15 to 25 percent of all the pullets housed succumb to illness or parasitism before they complete their first year of egg production. Worm infestation or other systemic disorders is responsible for poor feed conversion, loss of body weight, depressed egg production and an excessive number of culls.

Profits on a poultry farm can be increased, therefore, through control of disease and other causes of mortality. Excellent drugs and vaccines are available to the poultry farmer to combat these losses. However, prevention of disease is more economical than medical treatment after diseases have developed.

Disease organisms or parasites and their eggs may be found in the droppings or in the discharges from the eyes, nostrils and mouths of infected or carrier birds. The infective agent can be picked up by a flock from the litter, feed or water that is contaminated with the discharges of the sick birds. Outbreaks of parasitism or contagious diseases are believed to result from improperly maintained waterers, spilling of feed into the litter, or the contaminated condition of the litter itself. Water is the least expensive nutrient birds get and one that becomes contaminated

easily.

It seems reasonable to expect that certain parasitic and infectious diseases could be prevented or their incidence lessened by maintenance of a healthy environment and sound management practices. From the poultryman's standpoint, of course, it is necessary to find practical means of improving conditions in the hen house. With these thoughts in mind, the hypothesis was developed that perhaps disease incidence could be lowered through use of disinfectants in the drinking water and/or frequent introductions of clean litter.

Consequently, the purpose of the studies reported herein was to determine the effect of better sanitation practices on the mortality rate and egg production of laying hens fed diets that differed substantially in the character of certain ingredients. The sanitation and antibiotic practices used in these experiments are over and above those practiced in many modern operations.

REVIEW OF LITERATURE

According to Winton (1932), a carefully planned sanitation program on a poultry farm would aid in the control and eradication of certain infectious diseases and parasites. He came to these conclusions as the result of a practical experiment and flock survey in which litter was removed daily to avoid dampness and contamination. Winton cites an experiment conducted under farm conditions at Kansas Agricultural Experiment Station in which a flock of chickens given very little attention other than to provide food and water, had a total mortality of 42 percent throughout the year; whereas, a second flock, where the quarters were kept reasonably clean and sanitary during the year had an annual loss of only seven percent.

Barger et al. (1958) state that the health and performance of any flock depend mainly upon how closely one follows the principles of sanitation and good management rather than the use of medicines. The diseases of poultry are quite different in nature from those of other livestock. Of greatest importance are those considered mass disorders rather than ailments of individual birds. Therefore, the authors stress preservation of the health and vigor of the flock as a whole rather than devoting energies to a few individual sick birds.

Mortality in "contest" flocks

Dunnicliff (1913) in New South Wales analyzed mortality data from a total of 5,448 hens during a 10-year egg laying contest and reported a death loss of 6.3 percent a year. During the seventh Storrs Competition, Card and Kirkpatrick (1919) obtained mortality of 15 percent among 1,000 pullets. An average annual death rate of 25.4 percent was reported by Anderson (1928) during the first five years of the New York state egg laying contest at Farmingdale. In the above contest, the mortality was

28 percent among White Leghorns and 45.6 percent among Rhode Island Reds. Among 10,000 pullets entered in eight successive laying contests in Michigan, Stafseth and Weisner (1931) reported an average mortality of 19 percent a year. The fourth, fifth and sixth years of the contest showed an increase over the first three years. During the seventh and eighth years even a higher mortality occurred than in all previous years. More than 20 percent of the mortality during these years was due to laryngotracheitis, commonly called infectious bronchitis. Stafseth and Weisner further reported an increase in the incidence of peritonitis, sarcamatosis, bronchitis, pericarditis, tumors, leukosis, ruptured oviduct and salpingitis. On the other hand, they found decreased incidence of fowl cholera, tuberculosis, fowl typhoid and leg paralysis. An analysis of mortality data throughout the year showed a rather marked seasonal shift in the mortality curve. Highest mortality was observed in the months of March to July. The death loss in December and January was higher than that in November. The authors are of the opinion that higher death rate follows heavy egg production.

During seven successive egg laying contests in Utah, Alder (1934) obtained mortality percentages of 13, 35, 33, 27, 18, 20, and 58, respectively. He attributed the higher death rate in the last year to an outbreak of infectious laryngotracheitis. Dudley (1928) during a period of 15 years found a death loss of 7.2, 6.8 and 5.7 percent for White Leghorn, White Wyandottes and Rhode Island Reds, respectively. These breeds showed a different pattern of mortality during the year. The death rate of White Leghorn and Rhode Island Reds was similar during December, although it was considerably different in magnitude. From January to July the death rates of White Wyandottes seemed behind that of the other two breeds. The maximum death rate occurred in August with the White Leghorns, September with the White Wyandottes and March with the Rhode Island Reds. Mortality

rates of the White Leghorns and of the Rhode Island Reds reached a high level in March, fell to a low level in April, rose again in May and fell in June and then both increased.

Harris and Boughton (1927) have studied for a period of ten years the death rate among the birds entered for the International Egg Laying Contest conducted at Storrs, Connecticut. The death rate of White Wyandotte fowl during the first year of lay was significantly higher than that of the Rhode Island Red and White Leghorn fowl, with the former somewhat higher than the latter. They noted that death loss in different breeds was not the same during each month of the year, but showed a gradual increase from November to July. In all these breeds the death rate for May was lower than that for April. Harris and Boughton (1927), like Stafseth and Weisner (1931) expressed the opinion that higher death loss follows heavy egg production. They concluded that the strain of heavy egg production tends to eliminate the weaker birds. Harris and Boughton attempted to show a correlation between the monthly death loss and the average monthly egg production of each breed.

Brunson and Godfrey (1952) summarized autopsy records of the Oklahoma egg laying tests for a period of 14 years. They found an annual mortality rate of 13.84 percent to 26.3 percent with a 14-year average of 20.27 percent. Death rate among White Plymouth Rocks and White Leghorns was higher than that of New Hampshire or Rhode Island Reds. Major causes of mortality reported were reproductive disorders, lymphomatosis and respiratory infections.

New York random sample test (1953) report showed an average annual mortality of 35.5 percent in hens hatched as chicks. Lymphomatosis was responsible for 14.5 percent of this loss, two-thirds of which was caused by the visceral form of the disease.

Random Sample test report XX (1963) showed an average mortality rate of 10.86 percent among laying hens. The minimum and maximum death loss ranged between 0.00 and 30.60 percent.

Mortality of Young Chickens

Voorhies and Read (1931) made an extensive study of daily mortality of 6,000,000 chicks for the first 14 days of the brooding period in the years 1927, 1928 and 1929. Their data showed highest mortality on the fifth day, then a rapid fall off from the 6th to the 14th day, after which the rate of loss was again at the same level as on the firstday. Barrett (1929) studied the causes and distribution of mortality among a total of 4,806 chicks in seven hatches for a period of the first eight weeks and found a total loss of 11 percent. Voorhies and Read (1931) have reported a relatively high mortality, unassociated with any disease.

Broadfoot et al. (1957) reported an outbreak of infectious bronchitis in 14,000 susceptible pullet chicks in 35 flocks during the first 5 to 7 days of life. The chicks of high-producing strains suffered death losses varying from 5 to 35 percent.

Newcastle disease virus during the period of active involvement restricted feed and water intake and depressed weight gains of White Leghorn cockerels (Squibb, 1961). Vitamin therapy did not affect weight gains, water intake, feed efficiency or mortality of cockerels.

Hays and Spear (1952) determined that chick mortality from young parents was significantly higher during the first eight weeks of life than was observed in chicks from older parents. The data collected by Hays (1955), over a 5-year period on pedigreed Rhode Island Reds bred for high fecundity did not indicate that age of parents significantly affected either viability of pullets in the laying house or the viability of males and females during the growing period.

Dardiri and Zaki (1955) reported oomphalitis in 135,000 chicks shipped from New York to Cairo, Egypt. Highest mortality occurred on the second to sixth day of infection. Pseudomonas aeruginosa, Proteus morgani, Aerobacter aerogenes and clostridia species were isolated from dead chicks.

Reid et al. (1961) isolated Escherichia coli, enterobacteriaceae, clostridium, Pseudomonas, streptococcus and Bacillus from dead embryos of chicks involving 25 hatcheries. E. coli isolated varied in pathogenecity showing from 20 to 100 percent mortality in baby chicks.

Chute and Mear (1957) considered litter as the source of infection in most cases of aspergillosis. Wright et al. (1960, 1961) successfully transmitted aspergillosis to incubating embryos which resulted in infected chicks. Penetration of sound and cracked eggs by Aspergillus fumigatus occurred within six days of incubation. He further added that the presence of certain molds, particularly Aspergillus species markedly increased chick and pullet mortality during the first ten days of life. Todd (1948) examined 1014 chicks for parasitic worms and found 972 (95.8 percent) parasitized by an average of 125.6 worms representing an average of 3.5 species. Tyzzer et al. (1932) reported that young birds were less susceptible than older birds to Eimeria necatrix. Contrary to this, Brackett and Bliznick (1952) found that following inoculation of equal number of oocysts, young birds were found more severely affected than old birds. Gardiner (1955) was of the opinion that chicks were most resistant to cecal coccidiosis at two weeks of age than at any other period during the first six weeks of their life. Benzanson and Stephenson (1948) reported that an increase in depth of litter greatly reduced the incidence of breast blister conditions in broilers. Wet litter tends to result in more breast blisters on broilers than dry litter (Smith, 1956).

According to recent reports from the Inspection Service, the greatest

single cause for condemnation during postmortem inspection is airsacculitis. Following close behind and on the increase is leukosis. Next in importance is septicemia or toxemia (Morris, 1963). Helmboldt et al. (1963) reported cutaneous lymphomatosis in nine-week-old broilers condemned for human use.

Mortality in Farm Flocks

Poultrymen who house their flocks in winter quarters at the right time, normally experience a low death rate in their flocks. Buster (1928) reported 24 percent mortality in 35 flocks having a total of 65,264 hens in the Petaluma district of California in 1926.

During a period of three years, 1926 to 1928 in Oregon, there was an average death loss of 13 percent among flocks totaling 271,337 hens (Scudder et al., 1931). Thomas and Clawson (1933) in Utah noted a period of three years -- 1929 to 1931, a mortality of twenty percent in 315,577 Leghorn hens kept in commercial flocks of about 1,000 each. Misner (1932) recorded 27 percent mortality on 108 New York State poultry farms during the years 1930-1931. The average number of hens per farm in this group was 959. The average mortality by years was 19, 24, 27, 37, 36 and 39 percent.

Darcel et al. (1960) determined 13 and 16 percent mortality over a period of two years in a White Leghorn flock of 9,442 birds. They observed a very low incidence of neoplastic diseases in general, and leukosis in particular. Most prevalent disease reported in this flock was histomoniasis. Takashi and Reid (1961) also made a survey and found an increasing trend of histomoniasis in chicken flocks.

Jaquette and Fogg (1962) posted 5,816 chickens from 1145 flocks and concluded that CRD complex was most widely spread disease on the Del Marva Peninsula. The other most prevalent diseases reported were coccidiosis and worm infestations. Other causes of mortality diagnosed were lympho-

matosis, synovitis, enteritis, hemorrhagic syndrome, laryngotracheitis, avian encephalomyelitis and mycosis.

Olesiuk and Van Rockel (1960) reported that CRD caused a rapid decline in egg production and long-lasting effects on both external and internal egg quality. The egg abnormalities reported were rough and thin shelled, bleached shell color and watery albumen. Raggi et al. (1961) found that infectious laryngotracheitis had only temporary effects on egg production and shell thickness. Taylor et al. (1955) reported an outbreak of avian encephalomyelitis in a breeding flock of chickens. Eggs produced just before and during the period of depressed egg production showed decreased hatchability and an increased embryonic mortality during the last three days of incubation.

Prolapse in the chicken is defined as the eversion of the oviduct with the cloaca through the vent (Barger and Card, 1935). Stafseth et al. (1932) suggested that prolapse may result from atony associated with toxic action of products incident to worms, coccidia or secondary microbial invaders. Wheeler and Hoffmann (1948) reasoned that prolapse and pickouts in chickens may be either a sudden increase in estrogens in the blood or a protracted high estrogen blood level. Contrary to this hypotheses, Burmester (1948) found that females given estrogen (diethylstilbestrol) had the lowest incidence of prolapse although not statistically different from the controls. Neal (1956) reported that methionine when supplemented at a higher level suppressed cannibalism and pickouts.

Gross (1961) isolated from litter and feed samples, Escherichia coli pathogenic serotypes. Calnek and Levine (1957), Fabricant et al. (1959) and Gross (1961) found these serotypes associated with mycoplasma (pleuropneumonia-like organisms or PPLO), infectious bronchitis virus (IBV) and Newcastle disease virus (NDV) in cases of air sac disease complex. Olesiuk

and Van Rockel (1960) reported that chickens may become infected with pleuropneumonia organisms (PPLO) by egg transmission or by close contact with infected birds. Van Rockel (1955) was of the opinion that environmental factors such as over-crowding, low or irregular brooding temperature may increase the transmission and severity of disease. Calnek and Levine (1957), Olesiuk and Van Rockel (1960) reported that viruses such as IBV and NDV can greatly increase the transmission and severity of PPLO infection.

Chalquest and Fabricant (1959) suggested the possible control of the transmission of pleuro-pneumonia-like organisms by dipping hatching eggs into cold antibiotic (erythromycin) solution.

Waters and Prickett (1944), Waters and Bywaters (1949) suggested egg transmission of leukosis virus which was supported further by Gross (1951, 1952), Cottral (1952) and Cottral et al. (1954). Contrary to this hypothesis, Hutt et al. (1944), Cole (1949), Cole and Hutt (1951) and Hutt (1951) found that some forms of lymphomatosis were not egg transmissible. Patterson et al. (1932), Kennard and Chamberlin (1934) successfully transmitted lymphomatosis in groups of chicks inoculated with nasal washings from both infected and normal mature hens.

Burmester and Waters (1955) are of the opinion that lymphomatosis virus which is deposited in eggs laid by hens having a latent infection, is transmitted to the embryo and chicks which hatch from such eggs. These maternally infected chicks may be a source of infection for chicks from other parents not similarly infected and hence are lacking in antibodies. They further state that the transfer of such infection appears to take place during the brooding period. Burmester et al. (1955) found 17 of the 22 hens of the infected population shedding virus into their eggs.

Cole and Hutt (1956) reported variations in the amount of lymphomatosis virus in the eggs of the same hen. Burmester and Waters (1956) found a

decrease in the shedding of the lymphomatosis virus into eggs as hens became old. Burmester and Gentry (1954) produced lymphomatosis in birds given the virus via the trachea (83%) or as an aerosol spray (39%). Sevoian et al. (1963) determined that lymphomatosis agents can be transmitted in birds via the air route. The clinical and pathological manifestation of the disease were undistinguishable from those of parenterally induced or natural infection.

Sevoian and Chamberlin (1963) and Cole and Hutt (1954, 1956) found that neural form was most prevalent in younger age groups (4-20 weeks) of chickens and visceral form in mature birds. Burmester et al. (1959) reproduced the visceral form of lymphomatosis after long incubation periods. Neural form was rarely observed by these workers. However, it does not necessarily follow that each form is caused by a different agent. They reported further that the age of the chicken and variation in the dose of virus at infection time is a factor influencing the response of the chickens to tumor viruses. High doses primarily caused erythroblastosis, which occurred in less than four months and low doses resulted in a response that was primarily visceral lymphomatosis, with death occurring after four months. They further state that filtrates of tumors and other materials from 5 of the 7 propagated lymphoid tumor strains caused osteopetrosis in addition to erythroblastosis and visceral lymphomatosis.

Kennard and Chamberlin (1936) and Hutt and Cole (1954) indicated that assistance to leukosis increases with age. Sevoian and Chamberlin (1963) reported that day-old chicks were approximately 1,000 to more than 10,000 times as susceptible as their dams and grand dams. Waters (1951) collected data over a nine-year period on resistance and susceptibility to lymphomatosis among six different inbred lines of White Leghorns maintained for a period of 600 days. He reported a marked increase or decrease in the percentage of

mortality from lymphomatosis resulted in certain lines where inbreeding accompanied by rigid selection was practiced. Hutt and Cole (1954) reported that the severity of exposure increases with the proximity of the susceptible chicks to adult hens infected with leukosis. They further state that chickens raised in batteries contracted leukosis just as readily as did other chicks on the floor.

Kennard (1933) found the mortality from neurolymphomatosis in hen-bred pullets about half that of pullet-bred pullets. Contrary to these results, Carr (1952) and Cole (1955) found that pullet-bred families were free of death from lymphomatosis. During the next year, Cole and Hutt (1956) reported that in pedigreed flocks bred for resistance to leukosis, age of the hen had little effect on the resistance of her daughter.

Forgacs and Carll(1955) and Forgacs et al. (1954) isolated toxic fungi (Aspergillus clovatus, A. flavus, and A. Fumigatus) from feed and litter samples collected from areas where poultry hemorrhagic syndrome was enzootic. Using fungi, which they isolated from litter samples, Forgacs and Carll (1955) produced clinical and pathological conditions of mycotoxicosis in chickens similar to those found in field cases of hemorrhagic syndrome. Later, these findings were confirmed by Forgacs et al. (1955, 1958, 1962) who produced toxicosis in chickens housed both in batteries and floor pens. The addition of vitamin K to the toxic fungal diets did not prevent the mycotoxicosis in chickens.

Enigh (1935) reported sixty-one cases of blackhead in German flocks caused by "Blastocystis" fungus, later reported to be yeast-like fungus condida albicans.

Couch (1955) and Snoeyenbos et al. (1961) found an increase in the incidence of cage paralysis with the rapid increase in the practice of keeping layers in individual cages.

Reports from various diagnostic laboratories (Snyder et al., 1962) indicate that leukosis, coccidiosis, chronic respiratory disease and ascariaris are the most prevalent diseases among farm flocks. Other causes of mortality in chickens reported are avian monocytosis, enteritis, synovitis and capillaria worms.

Bactericidal Effect of Chlorine

One to two ppm of chlorine (Anon., 1949) destroys not only bacteria but also viruses. According to Ehleres and Steel (1927) chlorine possesses selective bactericidal effect, destroying bacteria mostly of the pathogenic and coli groups. A few harmless bacteria and spores are rather resistant to its action. Ehleres and Steel (1927) stated that chlorine can be applied to water, either as gas or in the form of chlorinated lime. Chlorine gas is preferable to chlorinated lime because it does not lose its activity as readily as the latter and is more convenient to handle. Chlorinated lime contains 30 to 35 percent by weight of available chlorine.

Disinfection by chlorine (Anon., 1949) depends on the concentration of residual chlorine, the temperature of the water, and the amounts of organic impurities present in it. A reasonably higher temperature and acidic media favor bactericidal action. Ehleres and Steel (1927) found that in water containing little or no organic matter, 0.2 ppm of available chlorine is sufficient to kill bacteria. According to them, chlorine in water forms unstable hypochlorous acid which readily breaks down into hydrochloric acid and atomic oxygen which possess bactericidal property. The orthotolidine test, when properly applied, measures the amount of residual chlorine.

Deep Litter

Deep litter, if properly managed, can keep the house warm and dry. As described by Lippincott and Card (1946), the usual practice with deep or

built-up litter is to start in the fall with 2 to 4 inches of dry litter, and then add more gradually until the floor is covered 8 to 12 inches deep by about the middle of December. After this no more litter is added, but the upper surface is stirred occasionally to prevent matting. The litter should be spread evenly on the floor, and wet cakes formed around the water fountains removed and replaced with new, dry material. It may be removed and replaced with clean, shallow litter in the late spring or it may be kept until the regular fall cleaning.

Effect of Deep Litter on Incidence of Mortality

A number of research workers have discussed highly absorbent and insulating properties of deep litter. According to Kennard and Chamberlin (1949), as well as Moore and Chamberlin (1953), the heat generated by the microbial decomposition of the litter has a self-sterilization effect. Biological and chemical reactions in this type of litter can destroy harmful bacteria, protozoa and other infective agents. Kennard and Chamberlin (1948b, 1949, 1950a, 1950b and 1951) reported decreased incidences of cannibalism, cecal coccidiosis and pullorum disease in birds maintained on built-up litter. They reared 26 consecutive broods of chickens on the same litter and found that whether limed or unlimed, it contained fewer yeasts, molds and bacteria than new litter changed frequently. Koutz (1952), however, demonstrated that coccidial oocysts were not killed in deep litter. Boughton (1939) advocated the use of deep litter as one method for control of coccidiosis in young chicks. He found that rapid drying or extreme wetness reduced the number of sporulating oocysts; a limited amount of moisture was highly desirable for microbial action and composting of the litter and droppings.

Contrary to the results of the previous investigators, Koutz (1952, 1953) found that all birds on built-up litter were heavily infested with

parasites such as Eimeria tenella, Eimeria acervulina, Eimeria maxima, Ascaridia lineata, Heterakis gallinae and Capillaria retusa. He made periodic checks on this litter and found numerous ova and oocysts of these parasites. The control birds were free from intestinal nematodes although some coccidia were present in a few birds.

Klein of the University of Massachusetts (Hinshaw, 1952) felt that deep litter increased the flea problem in poultry houses. Rats, cats and dogs were blamed as constant reservoirs of these fleas. Cotterill and Winter (1953) reported that litter which becomes too wet may result in excessive ammonia production and impair the health of the poultry flock. Faddoul and Ringrose (1950) observed that avian keratoconjunctivitis in young chicks developed as results of ammonia liberated from built-up litter.

Addition of Lime in Built-up Floor Litter

The use of built-up litter systems became a well established practice in the late forties. Subsequently, lime was added to the litter to avoid dampness. The New Jersey Agricultural Experiment Station reported that hydrated lime could be used safely as a deodorizer and preservative of nitrogen in bird droppings. Lime was recommended for floor litter (Kennard and Chamberlin, 1947) in brooder houses as a control measure against the spread of coccidiosis. They cite an experiment at Western Washington Agricultural Experiment Station where the use of lime in floor litter was found favorable.

When liming litter, hydrated lime is scattered over the litter at the rate of 12 to 15 pounds per 100 square feet of floor space or one pound per layer. The newly added lime is covered with 1" to 2" of fresh litter. Both are stirred into the old floor litter at intervals of 2 to 4 weeks. Moore and Chamberlin (1953) emphasized that, depending upon the weather and local conditions, care must be taken to keep litter dry by frequent stirring.

Deep Floor Litter as Source of Dietary Supplements

Hammond (1942) and Whitson et al. (1945) reported that cow manure, when added to a diet deficient in riboflavin, had marked beneficial effects, as measured by livability of young embryos, hatchability of eggs and rate of chick growth. Rubin et al. (1946) and Bird (1947) demonstrated that chick growth factors in cow manure were also present in the droppings of hens. Kennard and Chamberlin (1948a, 1948b) showed that deep litter was a potent source of dietary supplements including the unidentified animal protein or vitamins necessary to supplement an all-plant protein diet for higher egg production and hatchability.

Halbrook (1950a) isolated 140 different organisms from built-up litter capable of synthesizing vitamin B_{12}. During the same year in another publication, Halbrook (1950b) and Halbrook et al. (1950) reported 250 mu of vitamin B_{12} per gram of built-up litter as compared to 1 mu in unused control litter. Kennard and Chamberlin (1951) found that there was a direct correlation of the rate of growth, feed efficiency, and rate of mortality with the age of the floor litter, whether the chickens received the all-plant diet with or without supplements.

Experimental evidence has been accumulating during the past thirty years to establish the fact that the synthesis of vitamin or vitamin-like factors is accomplished after the feces are voided. Lamoreux and Schumacher (1940) reported that the feces of laying hens contained no more riboflavin at the time of defecation than was present in the feed which the hens were receiving. McGinnis et al. (1947) and Lamoreux and Schumacher (1940) found a 100 percent increase in the riboflavin content of feces when held at room temperature for 24 hours and a 300 percent increase when held for one week. The authors were of the opinion that riboflavin synthesis did not occur in the digestive tract of the fowl but resulted after the feces were voided.

Crowley et al. (1961), Marr et al. (1961) and Singsen et al. (1961) reported that the calcium-phosphorus requirement is higher for laying hens maintained in cages than for those maintained on litter in floor pens. They suggested perhaps hens housed on litter may acquire part of their calcium-phosphorus requirements through coprophagy.

Effect of Deep Litter on Hatchability

Kennard and Chamberlin (1948a) reported 78 percent hatchability of eggs from hens that were on old, built-up litter and that received an all-plant diet versus 32 percent hatchability of eggs from the same ration given to similar hens on litter that was removed and renewed every two weeks.

Sunde et al. (1951) also found that the hatchability of eggs from birds on a basal, all-vegetable diet increased with the age of the built-up litter.

Intestinal Flora of Chickens

Kern (1897), King (1905) and Gage (1911) have reported that Escherichia coli and Aerobactor aerogenes are the predominant organisms of the intestinal flora. E. coli is a constant inhabitant of the intestinal tract and tends to replace other organisms whose presence depends upon the food supply. King (1905) and Gage (1911) further reported the presence of Lactobacilli, Micrococci, pseudomonads, bacilli, Sarcinae clostridia and yeast in the chick intestines.

Bacterial Flora of Chicken Droppings

Emmel (1930) reported that the bacterial flora of the feces was approximately the same in both chickens and hens -- Escherichia coli and Escherichia Communior were the predominant organisms in the feces of both two-week-old chicks and of hens. Schumacher and Heuser (1941) have demonstrated the presence of E. coli along with sporeformers, non-gelatin liquifiers, gram-positive cocci, molds and Bacillus Megatherium in chicken feces.

The Microflora of Droppings and Its Relation to Litter Management

Halbrook et al. (1951) have suggested that the microflora of fresh droppings has a trend similar to that of the litter on which the chicks run. They obtained lower counts from droppings of chickens on built-up litter than from droppings of those kept on litter that was changed every eighth week. They further stated that the droppings of birds on wire floor have counts as high as those of chicks on litter. The authors were of the opinion that the flora in birds was mainly established through feed and water and suggested that perhaps alkalinity of built-up litter had a depressing effect on the intestinal microflora of the bird.

Halbrook et al. (1951) have reported an increase in molds and yeast with the age of the litter for the first eight weeks. Cob litter used for chick brooding but changed weekly contained more bacteria, mold and yeast than new cobs; litter unchanged for eight weeks contained more than the weekly changed litter. Built-up litter which had been in use for over one year contained fewer yeast, molds and coliform bacteria than either weekly-changed or unchanged litter. These researchers have also made counts on the bacterial population of litter used for chick brooding for eight weeks and found a hundred times more molds and yeast, fifty times more coliform, fifteen times more Lactobacilli than was found in built-up litter. However, total counts between unchanged and built-up litter did not vary to a great extent. The aerobic bacterial count was higher in built-up litter than in new, unused litter by 1,000 to 2,000 times. They reported coliform and yeast counts higher in built-up cob litter than in new cobs.

EXPERIMENTAL PLAN AND STATISTICAL PROCEDURES

Experimental Plan

The study reported herein includes ten experiments conducted over a period of two years. The report is divided into two main parts with the first consisting of eight experiments on chlorination; the second part consisting of two experiments relating to better sanitation practices.

A preliminary study of chlorination of drinking water was carried out to determine how much chlorination chickens can tolerate without curtailing water intake and performance. Growing chicks and laying hens of different breeds and varieties were supplied chlorinated water with chlorine levels ranging from 25 to 2,000 ppm.

Experiments with commercially-hatched Leghorn-type hens were undertaken in order to determine the effects of better sanitation practices on egg production and rate of mortality.

Statistical Procedures

Data from the experiments were subjected to statistical analysis. Data for individual birds were analyzed by the analysis of variance as outlined by Snedecor (1956). The standard error of the mean was then calculated and Duncan's (1955) multiple range test employed to determine which means were significantly different at both the .01 and .05 levels of probability. Egg production and mortality data for treatments are expressed in percentages, but the actual number of eggs each hen laid over the period was subjected to analysis of variance and Duncan's multiple range tests. The arc sine transformation of mortality data was carried out before it was subjected to analysis of variance and Duncan's multiple range tests.

PART I

INTRODUCTION TO EXPERIMENTS I THROUGH VIII

Water is an important nutrient which may easily become contaminated with bacteria, viruses, protozoa and algae (Fair et al., 1954). These may sometimes be responsible for an outbreak of disease in poultry. The presence of these organisms in the bird's water supply seems objectionable and consequently their destruction or prevention of growth may be of some significance for flock owners, but convincing proof of their causation of disease under practical conditions is lacking.

Chlorine at levels from 1 to 2 ppm in water is not only bactericidal but also destroys viruses (Anon., 1949). Thus chlorination of water might prevent the spread of any contagious water-borne infections. There is very little information at present as to how much chlorinating chickens can tolerate without affecting water intake and performance. Such information would be necessary if the poultry industry is to take advantage of chlorinated drinking water for the improvement of poultry sanitation.

Having this idea in mind, chlorinated water of different concentrations was offered to experimental birds. Data collected were gain or loss in body weight, feed intake, water consumption, rate of mortality, and egg production. In Experiments III through VIII, group weights were taken and this explains why statistical analyses were not performed. These studies were conducted May through August using 25 to 2000 ppm of chlorine in water. A five percent solution of sodium hypochlorite was used as the source of chlorine. Available chlorine in water was tested by the orthotolidine reaction with the W and T*

* Wallace and Tiernan Comparator, Belleville 9, N. J.

comparator.

Before actual experimentation with birds, a laboratory study was undertaken to determine whether chlorine was available in filtrates from chlorinated water-feed mixtures. This was felt desirable because drinking water is usually contaminated to a greater or less degree by feed from the beaks of the birds. Varying amounts of the all-mash feed mixture (Appendix Table 1) was weighed in clean, dry Erlenmeyer 500 ml flasks and water having 500 to 1000 ppm of chlorine, was added in amounts as shown in Appendix Table 2. The flasks were shaken vigorously for three minutes and, at intervals, these mixtures were filtered. The filtrates were tested for residual free chlorine by the orthotolidine reaction. In order to get a filtrate from a mixture of feed and chlorinated water, the amount of water had to be double that of the feed. On shaking 50 grams of all-mash feed with 70 mls of water having 500 ppm of available chlorine, no residual chlorine was obtained at ten minutes. When the same amount of feed was shaken with 80 mls of water containing 1000 ppm of chlorine, the filtrate showed 50 ppm of chlorine after five minutes but there was none at 15 minutes. On shaking vigorously 32 grams of feed with 120 mls of water containing 1000 ppm of chlorine, the orthotolidine test revealed 250 ppm of chlorine at five minutes. This availability of chlorine decreased to one ppm within half an hour and there was no available chlorine at 45 minutes (Appendix Table 2).

PROCEDURE AND RESULTS

Experiments I - II. The Effect of Chlorination of Drinking Water on the Performance of Baby Chicks

Two experiments were conducted each with a different level of chlorination. One-day-old Single Comb White Leghorn cockerels were divided into equal weight groups and from these groups randomly distributed into the various experimental pens of chick starting batteries. Control replicates were offered tap water while experimental groups were given chlorinated water of different concentrations. Feed consisted of a standard chick starter (Appendix Table 1) fed ad libitum. Feed for each group was weighed at the beginning and end of the experimental periods and the feed consumption and mortality data recorded.

Stainless steel water troughs were attached to batteries. Each morning the troughs were thoroughly cleaned and refilled with chlorinated water for the rest of the day. For a period of one week, the unconsumed water in the troughs was weighed and the average water consumption per bird calculated. Water samples were collected daily in sterilized glass bottles containing a few crystals of sodium thiosulphate and taken immediately to the laboratory for plating on nutrient agar to determine total bacterial counts. An attempt was made to maintain chlorination at its initial levels by adding more chlorinated water twice a day.

Dead birds were taken immediately to the diagnostic laboratory for autopsy. As far as possible the cause of death was determined. On the day the first experiment was terminated, control birds as well as birds from groups where 1500 ppm chlorination levels in water were administered were killed. The intestines including ceca were transferred aseptically to sterilized glass bottles and taken immediately to a laboratory. The intestines were homogenized for two minutes in a sterilized blender with an amount of water double the amount of their weight. The homogenized material was made into a number of

of serial dilutions so as to have count ranges from 30 to 300 colonies per plate.

Agar Pour Plate Method Test -- The purpose of this test was to ascertain the total bacterial population of the intestinal tract. The basic media for the agar plate consisted of:

1. Bacto* - Agar, 15 grams
2. Bacto - nutrient broth, 5 grams
3. Distilled water, 1000 cc.

Autoclaved at 15 pounds pressure for 15 minutes.

Just prior to plating, the agar media was liquified by heating and cooled to 42° C and then poured over a measured quantity of intestinal material on the petri dishes. When the agar became solidified, the petri dishes were kept inverted in the incubator for 24 to 48 hours. At 24 to 48 hours the colonies were counted.

Experiment I: One hundred and fifty-four day-old cockerels were alloted to different groups and except controls were supplied different levels
of chlorination (100 to 1500 ppm) for a period of three weeks.

Experiment II: One hundred and twenty birds were selected at random from Experiment I and redistributed so that each lot had an equal number of birds with approximately the same live weight. Experimental groups were offered lower levels of chlorinated water (25 to 150 ppm) for a period of another two weeks.

The experimental design, body weights, feed and water consumption, as well as feed efficiency data are summarized in Tables 1-1 and 2-1, while the analysis of variance for these are shown in Tables 1-2 and 2-2. Significant differences ($P < .01$) were found in body weights of different groups when analyzed statistically. Rate of growth of control birds was significantly

* Available from Difco Laboratories, Detroit, Michigan

higher ($P < .01$) than experimental birds provided chlorine (250 ppm or more) in drinking water. Chlorination up to 150 ppm had slight but non-significant effect on growth rate and feed efficiency. Depression in growth increased as the levels of chlorine in water increased above 100 ppm.

During the third week three birds were found dead in each lot where 1,000 and 1,500 ppm of chlorine was made available in water. Autopsy on these birds did not show any lesion except emaciation and empty crops. The weights of heart, liver and gizzard were proportional to body weights (Table 1-3). Water samples were then plated on nutrient media. No growth of bacterial colonies was observed. Higher concentrations of chlorine in drinking water had a depressing effect on the bacterial flora of the intestinal tract (Table 1-4).

Table 1-1. Feed Intake, Water Consumption and Feed Efficiency of White Leghorn Cockerels With Different Levels of Chlorination of Drinking Water

Treatments	Chlorine levels	Average weights			Feed cons./bird		Feed/gain		Av. water cons./bird for 7 days
		1 wk.	2 wk.	3 wk.	0-2 wks.	0-3 wks.	0-2 wks.	0-3 wks.	
	ppm	gms.			gms.				gms.
A	0.3	88.5	151.	260.	240	497	2.12	2.22	420
B	100	89.0	147.	264.	237	465	2.17	2.25	392
C	250	80.0	145.	242.	231	470	2.16	2.28	371
D	500	85.0	141	226.	226	436	2.19	2.31	364
E	1000	65.5	107.	157.	160	306	2.25	2.30	252
F	1500	60.0	93.	121	121	191	2.20	2.30	210

Table 1-2. Analysis of Variance of Three-week Body Weights of White Leghorn Cockerels Used in Experiment I

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio	F .01
Total	147	498,080			
Treatments	5	396,576	79,314	111	3.02
Error	142	101,504	715		

Standard error of mean = 5.4

Treatments:

A	B	C	D	E	F
260	246.5	242	226.5	157	121

Means not underscored by the same line are significantly different ($P < .01$).

Table 1-3. Weights of Liver, Heart and Gizzard of Three-week-old Cockerels Sacrificed During Experiment I

Chlorine levels	Body wt.	Liver wt.		Heart wt.		Gizzard wt.	
		As-is	% Body wt.	As-is	% Body wt.	As-is	% Body wt.
ppm	gms	gms		gms		gms	
1500	105	3.0	2.8	0.5	.47	6.0	5.7
"	117	3.0	2.5	1.1	.94	7.2	6.1
"	144	4.0	2.8	1.0	.69	8.0	5.6
"							
"	174	5.0	2.9	1.5	.86	9.0	5.2
"	143	4.0	2.8	1.0	.70	6.4	4.4
"	182	5.0	2.7	1.2	.66	10.7	5.8
"	140	3.2	2.3	1.1	.78	7.0	4.9
"	116	3.0	2.6	1.1	.94	6.4	5.5
"	95	2.5	2.6	0.6	.50	4.6	4.8
"	88	2.5	2.8	0.6	.69	4.5	5.1
0.3	294	8.0	2.7	2.5	.85	12.0	4.1
"	292	8.0	2.1	2.1	.72	12.0	4.1
"	247	7.0	2.9	2.1	.85	11.0	4.5
"	265	8.0	3.0	2.1	.79	11.0	4.2
"	273	7.0	2.5	2.0	.73	10.0	4.0
"	242	7.0	2.9	1.9	.71	11.0	4.5
"	283	8.0	2.8	2.3	.81	12.0	4.2
"	246	6.0	2.4	2.1	.85	10.0	4.1
"	252	7.0	2.7	2.0	.79	11.0	4.4
"	268	8.0	3.0	2.1	.79	12.0	4.5

Table 1-4. Bacterial Population of Intestines of Three-week-old Cockerels Used in Experiment I

Chlorination levels	Body weight	Bacterial colonies/ml of intestinal material
ppm	gms	Thousands
0.3 (control)	292	6,000
"	247	100
"	294	2,000
"	273	640
"	265	6,000
"	242	4,000
"	246	2,000
"	283	500
"	252	4,000
"	268	6,000
"	243	8,000
"	287	45,000
"	251	760
1500	105	60
"	117	440
"	144	120
"	174	4,500
"	143	60
"	182	80
"	140	60
"	116	3,500
"	95	80
"	88	65
"	192	74
"	117	570
"	132	160

Table 2-1. Effect of Chlorination ofWater on Feed and Water Consumption as Well as Gain in Weight of Five-week-old White Leghorn Cockerels Used in Experiment II

Treatments	Chlorine levels	Average wt/bird (gms)		Feed cons./ bird	Feed gain	Average water cons/bird/day
	ppm	0-3 wks	3-5 wks	gms	3-5 wks	gms
A	0.3 (control)	222.0	418	600	3.06	162
B	50	222.5	420	604	3.02	160
C	100	222.5	417	570	3.19	152
D	150	222.0	396	551	2.17	130

Table 2-2. Analysis of Variance of Body Weights of Five-week-old White Leghorn Cockerels Used in Experiment II

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio	F .01
Total	119	620,483			
Treatments	3	11,012	3,671	.7	3.95
Error	116	609,471	5,254		

Treatments:	D	C	B	A
	396	417	420	418

Means underscored by the same line are not significantly different ($P < .05$)

EXPERIMENTS III, IV and V

The Effect of Chlorination of Water on Its Consumption by Growing Chickens

General procedures for the following experiments were the same, except that the chlorine levels in drinking water were varied. Experiment III was of 15 days duration while Experiments IV and V were continued for 35 days.

The chickens in different lots were weighed at the beginning and end of each experiment. The all-mash growing ration formula used is found in Appendix Table 3.

Feed was added to the hoppers as necessary to keep an ample supply before the birds at all times. The feed for each lot was weighed at the beginning and end of the experimental periods and the feed consumption and mortality data were recorded. To supply the chlorinated water, an inverted waterer of approximately four-quart capacity was placed in each pen of a battery. This type of waterer exposed a minimum of surface to atmospheric evaporation and contamination.

Each day a weighed quantity of chlorinated water was prepared and placed in each vessel; at the end of 24 hours, the unconsumed water was weighed and the daily water consumption per bird calculated. The evaporation was not taken into account because of the fact that only a very small area of water surface was exposed to atmospheric evaporation and all waterers had openings of the same dimensions. On alternate days, a water sample was collected in a sterilized glass bottle containing a few crystals of sodium thiosulphate. This water sample was immediately taken to the laboratory where it was tested for the presence of coliform and Salmonella organisms. Three times a day a water sample was taken from each waterer and tested for the residual free chlorine by the orthotolidine reaction.

Experiment III. -- One hundred fifty ten-week-old White Plymouth Rock chicks were distributed equally in five lots each being confined in a separate pen of a finishing battery. Levels of chlorination ranged from 25 to 100 ppm.

Experiment IV. -- One hundred twenty five-week-old Cornish Cross chickens were divided into six groups and except control were offered 100 to 500 ppm of chlorine in drinking water.

Experiment V. -- Seventy five five-week-old White Rock chicks were divided into five lots each alloted a separate pen of a battery. Chlorine levels in drinking water varied from 500 to 2,000 ppm.

Chlorine up to 75 ppm did not affect the daily water consumption or feed intake. There was a slight reduction in the daily water consumption of chickens at a level of 100 ppm of chlorine (Tables 3-1 and 4-1). There was a gradual decrease in water intake with increasing levels of chlorine above 100 ppm (Table 4-1).

From 100 to 1,000 ppm of chlorine decreased the water consumption but had no appreciable effect on the average daily gain in weight or the feed consumption per bird. Chlorine levels at 1500 and 2000 ppm in water decreased water and feed consumption as well as gain in weight. These results are shown in Table 5-1.

Water samples taken during each experiment 24 hours after watering were negative to coliform and Salmonella organisms. The chlorine levels in water after 24 hours depended upon the original chlorine levels as well as upon the amount of the feed added in the waterers by the beaks of the birds (Table 3-2).

Table 3-1. Chlorinated Water Consumption by Ten-week-old White Rock Chickens* During a Period of 15 Days

Chlorine levels	Water consumed/ bird/day	Feed consumed/ bird/day
ppm	gms	gms
0.3	147	110
25	145	111
50	148	109
75	148	111
100	134	109

* 30 birds in each group

Table 3-2. Free Chlorine Levels in Drinking Water After Standing 24 Hours in Drinking Fountain

Initial chlorine levels	Chlorine levels after 24-hour period*
500	25
"	20
"	0
"	0
"	5
"	0
"	35
"	50
1000	150
"	100
"	10
"	20
"	600
"	100
"	100
"	500
1500	100
"	800
"	1000
"	20
"	800
"	500
"	1000
"	100
2000	200
"	1000
"	1200
"	10
"	1200
"	1500
"	500
"	100

* Variable amounts of feed were found in water troughs. Possibly this might have been responsible for lower levels of chlorine in water.

Table 4-1. Feed Intake, Water Consumption and Gain in Weight of Five-week-old Cornish Cross Chickens* During a Period of 35 Days

Chlorine levels	Gain in wt/ chick/day	Av. feed intake/ chick/day	Av. water cons/ chick/day
ppm	gms	gms	gms
0.3	22.0	66	143
100	23.8	67	134
200	21.8	68	115
300	20.3	68	110
400	22.3	66	106
500	20.4	65	93

* 20 birds in each group

Table 5-1. Feed Intake, Water Consumption and Gain in Weight of Five-week-old White Rock Chickens* During a Period of 35 Days

Chlorine levels	Gain in wt/ chick/day	Feed intake/ chick/day	Water cons/ chick/day
ppm	gms	gms	gms
0.3	27.27	81	282
500	29.35	82	170
1000	27.42	79	144
1500	10.6	65	116
2000	4.9	38	113

* 15 birds in each group

EXPERIMENTS VI, VII and VIII

Effect of Chlorinated Water on Performance of Laying Hens

Three experiments were performed using different levels ranging from 25 to 2,000 ppm of chlorine in water. For each experiment, White Leghorn-type hens of approximately the same weight that had been in production for a period of five months were selected. Birds were confined individually in wire cages and fed an all-mash egg mash (Appendix Table 4). All birds were weighed at the start and end of each experiment. Feed and water consumption and mortality data were collected.

The interiors of the galvanized water troughs were covered with polyethylene sheets. More than enough water to satisfy daily needs was provided and unconsumed water from each group at the end of each day was decanted and weighed. Fresh chlorinated water was then provided for the night. Water evaporation was not taken into consideration because all watering troughs were of similar dimensions.

The feed hoppers were filled only about half full in order to keep wastage due to "billing out" at a minimum. Each group was given a weighed quantity of feed which was reweighed at the end of the experiment.

Eggs were gathered four times a day and marked for identification. After seven days the eggs were weighed individually.

Experiment VI -- Forty-eight hens of approximately the same weight were distributed at random in six groups. To each group a different concentration of chlorine in water varying from 500 to 2,000 ppm was made available.

Experiment VII -- Seventy-two hens were selected at random and distributed into four groups. Two groups were supplied drinking water with chlorine levels of 25 and 50 ppm. An equal number of birds served as control and were supplied with tap water.

Experiment VIII -- The groups of birds consuming water with 25 and 50 ppm of chlorine in Experiment VII were reversed.

RESULTS

Experiment VI -- Daily feed and water consumption per bird was reduced in all groups supplied chlorinated water. In fact, there was a gradual decrease in feed and water consumption as the levels of chlorine in water increased. This was reflected in body weight changes -- the birds consuming water containing chlorine, 1000 ppm or more, lost 15 to 17 percent of their body weight. Birds in all groups excluding the control, started to molt on the tenth day of the experiment. The severity of molt depended upon the concentrations of chlorine in water and the decrease in feed and water consumption with the increased levels of chlorine.

Egg production of birds receiving the chlorinated water was much lower than in the control birds. The average weights of the eggs in all groups for a period of one week before the experiment and during the experimental period was about the same. These results are shown in Table 6-1 and statistical analysis for this data are given in Table 6-2.

Experiment VII -- Daily feed and water consumption as well as egg production was not affected by 25 ppm of chlorine in water. When the chlorine levels were increased to 50 ppm, a decrease in feed and water intake was obtained. These results are shown in Table 7-1 and statistical analysis for egg production data are given in Table 7-2.

Experiment VIII. Chlorine at a level of 25 ppm increased daily feed intake or water consumption as well as egg production. On the other hand, chlorination at 50 ppm depressed feed and water intake as well as egg production. It appeared as if there was an improvement trend in the performance of birds consuming chlorinated water at 25 ppm (Table 8-1).

Table 6-1. Effect of Different Levels of Chlorination of Drinking Water on Feed and Water Consumption, as Well as Egg Production in Laying Hens During a 21-day Period

Group	No. birds	Chlorine in drinking water	Gms/bird/day consumed		Total loss in body wt.	No. eggs laid	Av. wt. per egg	
			water	feed			before expt.	at 3rd week
		ppm			lbs		gms	gms
I, II	16	0.3	106	120	.9	170**	54.5	54.2
III	8	500	74	96	3.0	25	53.5	*
IV	8	1000	71	86	5.6	23	54.5	55.0
V	8	1500	65	83	6.5	29	53.4	54.25
VI	8	2000	54	82	7.1	28	56.4	*

* Very few or no eggs were laid
** Eggs laid by Group I = 89
Eggs laid by Group II = 81

Table 6-2. Statistical Analysis for Eggs Produced by Birds Used in Experiment VI

Source of variation	Sum of square	Degrees of freedom	Mean square	F value	F .01
Total	675	39			
Treatments	385	5	77	9.1*	3.70
Error	290	34	8.5		

* Significant at $P < .01$

Treatments:

Chlorine levels in water (ppm)	.3	.3	1500	2000	500	1000
Av. eggs/hen	11.1	10.1	3.6	3.5	3.1	2.9

Means not underscored by the same line are significantly different ($P < .01$)

Table 7-1. Chlorinated Water Consumption, Feed Intake, Water Consumption and Egg Production for Laying Hens During a 3-day Period

Group*	levels in drinking water	Water cons/ bird/day	Feed cons/ bird/day	Total Loss or gain in in body wt.	No. eggs laid	Av. wt/ egg
	ppm	gms	gms	lbs		gms
I	50	145	59	- 1.1	46	55.0
II	0.3	160	93	+ .6	90	56.4
III	25	185	88	+ 1.4	87	57.2
IV	0.3	203	88	+ .6	104	54.5

* 18 birds in each group

Table 7-2. Analysis of Variance for Eggs Produced by Birds Used in Experiment VII

Source of variation	Sum of squares	Degrees of freedom	Mean square	F ratio	F .01
Treatments	209	3	69.6		
Error	319	41	7.8	8.9*	4.31
Total	528	44			

* Significant at P < .01

Treatments:

Chlorine levels in water (ppm)	.3	.3	25	50
Av. eggs/hen	5.8	5.0	4.8	2.5

Means not underscored by the same line are significantly different (P < .01)

Table 8-1. Chlorinated Water Consumption, Feed Intake, Water Consumption and Egg Production in DeKalb Hens During a 30-day Period

Group*	Chlorine in drinking water	Gms. consumed per bird/day		Loss or gain in wt.	No. eggs laid
		water	feed		
	ppm			lbs	
I	25	162	91	+ 3.2	60
II	50	119	84	+ 1.4	55

* 18 birds in each group

PART II

INTRODUCTION TO EXPERIMENTX IX AND X

Floor litter, for the comfort of chickens, has gone through an interesting evolution over the last thirty years. Before World War II, the usual practice was to remove old litter and add new litter to laying and brooding houses every week or so. The purpose of frequent change of litter was mainly to avoid dampness so as to prevent spread of coccidiosis and other infections.

As a result of a study made on 26 consecutive broods of chickens, Kennard and Chamberlin (1947, 1948a, 1948b, 1949, 1950b, 1950c, 1951) advocated the use of built-up litter over frequently changed litter. Boughton (1939) and Kennard and Chamberlin (1947, 1949) reported that deep litter was successful for the control of coccidiosis in young chicks.

On the other hand, Koutz (1952, 1953) found that all birds on "built-up" litter were heavily infested with parasites such as Eimeria tenella, Eimeria acervalina, Ascaridia lineata, Heterakis gallinae and Capilloria retusa. Faddoul and Ringrose (1950), Cotteril and Winter (1953) blamed "built-up" litter as a source of excessive ammonia production and cause of kerato-conjunctivitis in young chicks.

For some people, especially flock owners who are interested in the health of their flocks, the conception of "built-up" litter seems contrary to the principles of hygiene and sanitation. So, it seems that the problem of litter systems for a poultry house is still open for further research.

The idea of frequent removal of litter is closely associated with principles of sanitation. Therefore, a study with laying hens was undertaken in which both treatments, chlorination of water and frequent litter change, were employed singly and in combination.

The results of this experiment indicate that the use of chlorine in the drinking water and better management practices or both may not be advantageous over the usual tap water and "built-up" litter when judged on the basis of mortality. Since there was considerable variability of diets and/or chlorination, these might have affected the results of better sanitation practices, a study was undertaken the following year when treatments included: (1) "built-up" litter and (2) its frequent removal and replacement. Blood chemistry from birds kept on different litter treatments in Experiment IX was similar and, therefore, it was not repeated in Experiment X. The birds used in these experiments had been properly vaccinated against infectious bronchitis, fowl pox and Newcastle disease.

Management measures were followed in an attempt to keep the experimental birds in a cleaner, healthier environment. The criteria used for evaluation were incidence and rate of mortality, gain or loss in body weights, egg production, egg weight and egg shell thickness measured at regular intervals.

EXPERIMENT IX

The Effect of Chlorination of the Drinking Water and Frequent Change of Litter on Egg Production and Incidence of Disease in Laying Hens

At the beginning of the experiment, the floor, walls and other equipment such as water fountains and hanging feeders in the experimental pens were thoroughly cleaned, scrubbed well with two percent lysol solution and dried completely. Control pens had some fresh litter added at intervals of eight weeks. About a half-inch layer or approximately ten pounds of dry, soft wood shavings were spread on the floor in each experimental pen and after every third day this litter was removed and replaced by new litter. At the start of the experiment, all equipment used for handling the litter was new. The birds were held on top of the covered roosts by two wooden partitions during the change of the litter. Fresh litter was stored in an adjacent pen previously disinfected. Droppings and used litter were transported away from the poultry house.

The feed hoppers were of the hanging type. Wire guards were provided to keep the birds from getting into them or perching on top, or billing-out the feed. Water fountains, rather than open troughs were used to reduce exposure of the water to contamination. For two weeks before the treatment, the birds to be given treated water were accustomed to drinking chlorinated water from this type of water fountain with levels of chlorine from 5 to 15 ppm. On the day this experiment was started, chlorine levels in the treated pens were raised to 25 ppm and maintained at that point thereafter. At regular intervals water samples were collected for determination of residual chlorine by the W and T Comparator. When the chlorine levels in water were reduced to five ppm, the water fountains were cleaned and refilled with fresh chlorinated water. The control birds were supplied tap water. A foot pad soaked in a disinfectant solution was placed in front of the entrance to each experimental pen. This was to prevent the possible transmission of any

infective agent via the caretaker's shoes. Plastic sheets separated each treatment group to reduce possible contamination via dust.

Every second week, birds were examined by clinical as well as microscopic means to determine any subclinical infectious -- principally coccidiosis, tapeworms and round worms. Any diseased birds were kept in isolation to prevent infection of others. Birds dying during the experiment were immediately taken to the laboratory for necropsy in order to investigate the origin of any infection. All birds were trapnested and their eggs marked for identification.

Four-hundred 25-week-old Leghorn-type pullets were randomized into four groups and fed diets containing soybean meal, raw soybeans, cooked soybeans, or a combination of raw and cooked soybeans. These experimental rations are shown in Table 9-1.

Each feed was supplied to four replicate pens which were given the following treatments: drinking water chlorinated, litter changed frequently, both water chlorination and frequent litter change, tap water and "built-up" litter (control). On the day this experiment was started the droppings of birds were examined for presence of coccidiosis and worm infestation. Male birds housed in pens with these pullets were sacrificed and examined. No specific macroscopic or microscopic lesions were noted in the sacrificed birds. The coccidial oocysts and nematode ova from feces of birds were found not significant. Hence, no medical treatment was given to these birds before the experiment.

At the end of the experiment, blood was collected for the following laboratory tests: differential leukocytic count, plasma protein, coagulation time, urea nitrogen, and sugar. For differential leukocyte counts, the blood smears were stained by Wright's method and 200 white blood cells counted per field. The results are expressed as percentages. Five ml of fresh blood

was withdrawn in a centrifuge tube containing heparin and centrifuged. Plasma samples were run on an autoanalyzer* for the determination of sugar** and urea nitrogen**. Plasma proteins were determined with a Hitatchi Refractometer**. The plasma was stored at 4° C for three days before it was tested for coagulation time by recalcification of plasma as outlined by Caldwell (1957). The agglutination titre of serum against Salmonella O group D*** and E. coli antigens O and H was determined both by the tube and rapid slide tests as are reported by Mackie and McCartney (1950).

The data on egg production and mortality for the various groups are shown in Tables 9-2 and 9-4. Statistical analysis of this data are given in Tables 9-3 and 9-5. Different diets had a significant effect on egg production, both at the .05 and .01 levels of probability. The overall egg production was higher ($P < .01$) on the control diet but nonsignificant differences were obtained between the birds fed the experimental diets. There was a drastic drop in egg production with the hens which received the pelleted concentrate and whole grain. This occurred whether the soybeans were cooked or raw. These results are shown in Tables 9-2 and 9-6.

Different management treatments had no significant effect on the incidence of mortality. However, there appeared to be less (nonsignificant) mortality among the birds fed the basal diet. As shown in Table 9-4, a very high incidence of pickouts occurred among all lots of birds but the number was especially high among those lots fed the experimental diets. Ruptured yolk and/or peritonitis and leukosis ranked second and third, respectively of the causes of mortality.

---

* Technocon Auto Analyzer, Technical Instruments Corp., Chauncery, N.Y.
** These determinations were made by Dr. D. A. Schmidt and his staff of the Veterinary College.
*** Difco Laboratories, Detroit, Michigan

Final body weights of birds did not show any significant difference among different diets or management treatments. However, there appeared to be greater gain in body weights among birds fed the basal diet or kept on built-up litter. These results are shown in Table 9-7 and the analysis of variance for this data are given in Table 9-8. In general, poor fertility and hatchability were maintained by birds fed various experimental diets and given litter change or built-up litter treatments (Table 9-9). There was no difference due to diets and management treatments on average egg weight or egg shell thickness (Tables 9-10 and 9-11).

Sera from birds in all groups showed no agglutination with antigens of E. coli and Salmonella type O Group D (Tables 9-12, 9-13). Differential leukocytic counts among birds of each group were similar (Table 9-14). Blood coagulation times did not differ significantly (Table 9-15). Blood sugar levels were significantly higher ($P < .01$) among birds having both chlorinated water and frequent litter change. These results are shown in Table 9-16 and statistical analysis of this data are given in Table 9-17. Plasma protein and blood urea nitrogen showed little variation among birds of different groups (Table 9-18 and 9-19).

Table 9-1. Percentage Composition of the Diets Used in Experiment IX

Ingredient	1	2	3	4
Soybean oil meal, 45% protein	21.4	--	--	--
Soybeans, whole raw	--	25.9	12.95	--
Soybeans, whole cooked	--	--	12.95	25.9
Fat, animal	4.50	--	--	--
Corn, ground yellow	59.9	--	--	--
Corn, whole grain yellow	--	59.9	59.9	59.9
Fish meal with solubles, 50% prot.	2.0	2.0	2.0	2.0
Meat and bone scraps, 50% prot.	2.0	2.0	2.0	2.0
Alfalfa leaf meal, 20% prot.	2.50	2.50	2.50	2.50
Limestone, ground	1.64	1.64	1.64	1.64
Dicalcium phosphate	1.20	1.20	1.20	1.20
Salt, iodized	0.25	0.25	0.25	0.25
Vitamin trace mineral mix*	0.25	0.25	0.25	0.25
Antibiotic**	+	+	+	+

* Nopcosol M-4, Nopco Chemical Co., Harrison, New Jersey.
** Lincomycin at 50 gm/ton

Calculated analyses:

Ingredients	%	Vitamins	Amt/lb
Protein	17.67	A IU	4021
Fat	7.27	D_3 added ICU	750
Fiber	3.42	B_{12} mcg	6.26
Calcium	3.0	Riboflavin mg	2.37
Phosphorus	.68	Niacin mg	19.89
Arginine	1.02	Pantothenic acid mg	5.79
Glycine	.90	Choline mg	537.00
Cystine	.25	Prod. energy Cal/lb	983
Methionine	.35	Folic acid mg	.23
Lysine	.90		
Tryptophan	.19	Calorie-protein	55.5

Table 9-2. Egg Production Percentage With Respect to Diet and Management Treatment

Diet	Control (C)	Litter change (L)	Chlorine in water (W)	Litter change and chlorine in water (CL)
1	75.5	72.7	66.0	70.0
2	58.4	46.1	49.8	50.0
3	48.2	53.4	57.7	53.3
4	42.2	43.7	62.5	65.5

Diet	1	2	3	4
Average value (%)	71.0	51.0	53.1	53.4

Treatments	C	1	W	CL
Average value	56.1	53.9	59.0	59.7

Means underscored by the same line are not significantly different ($P < .01$)

Table 9-3. Analysis of Variance of Egg Production Data With Respect to Diet and Management Treatments (168-day trap period)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F ratio	F .01
Diets	32,196	3	10,732	25.1	3.88
Treatments	207	3	69	.16	3.88
D X T	7,319	9	813.20	1.9	1.92
Error	99,296	232	428		
Total	139,018	247			

Diets	1	2	3	4
Av. values (%)	71.0	51.0	53.1	53.4

Management treatments	C	L	W	LW
Av. values (%)	56.1	53.9	59.0	59.7

Means not underscored by the same line are significantly different ($P < .01$)

Table 9-4. Incidence and Cause of Mortality Under Various Diets and Treatments

Diet	Treatment	% Mortality* (less pickouts & heart puncture)	Postmortem finding** (cases)									
			P	FC	S-T	RY-P	N	FD	L	I-O	X	S
1	Control	8.4	1								1	1
	Litter	3.7	3					1				1
	Cl. H_2O	6.7		1					1			1
	Litter - Cl. H_2O	4.2	2						1			
2	Control	16.7	18			2			1			
	Litter	7.4	4			1	1					
	Cl. H_2O	8.0				2						
	Litter - Cl. H_2O	8.3	6		1	1					1	2
3	Control	25.0	4			1	1		1	1		
	Litter	7.4	19			1	1				3	3
	Cl. H_2O	8.4	2		1	1						
	Litter - Cl.H_2O	12.5	3			1	1					1
4	Control	4.2	8		1							
	Litter	4.2	4							1		1
	Cl. H_2O	12.5	5		1	1		1			1	3
	Litter - Cl. H_2O	4.2	10			1					1	1
		Totals	89	1	4	12	4	2	4	2	7	14

* Nonsignificant at P .05

** Key to findings:

P = Pickouts
FC = Fowl cholera
S-T = Sinusitis and Tracheitis
RY-P = Ruptured yolk and peritonitis
N = Nephritis
FD = Fatty degeneration of liver
L = Leukosis
I-O = Impacted oviduct
X = Other nonspecific cases
S = Sacrificed for laboratory procedures

Table 9-5. Analysis of Variance of Mortality Data for Birds in Experiment IX

Source of variation	Sum of square	Degrees of freedom	Mean square	F ratio	F .01
Treatments	110.52	3	36.84	2.3	6.9
Diets	142.89	3	47.63	3.01	6.9
Error	142.20	9	15.8		
Total	395.61	15			

Diets	1	2	3	4
Av. value (%)	13.7	18.2	20.8	14.02

Management treatments	C	L	W	LW
Average value (%)	13.6	17.2	15.2	20.7

Means underscored by the same line are not significantly different ($P < .01$)

Table 9-6. Percent Egg Production Under Various Diets and Management Treatments Computed on basis of Total Number of Live Birds Present at the Beginning of Each Month

Diet	Treatment*	Dec.	Jan.	Feb.	Mar.	April	May
1	L	75.2	82.1	70.0	71.1	70.1	68.0
	W	57.6	68.0	64.3	72.4	66.9	66.2
	WL	70.6	72.0	70.2	69.4	67.6	66.2
	C	71.4	64.0	67.5	81.1	84.0	84.2
2	L	45.6	47.9	35.7	44.1	58.6	64.7
	W	44.6	41.2	47.0	40.1	64.6	61.3
	WL	43.9	32.8	28.2	60.1	66.7	68.4
	C	94.8	36.6	11.3	20.8	57.7	90.3
3	L	42.8	33.8	30.5	39.9	83.3	90.3
	W	64.3	54.5	48.6	54.2	59.5	65.0
	WL	55.0	47.5	47.5	52.2	57.4	60.1
	C	48.6	41.2	28.2	55.1	58.2	58.0
4	L	53.7	41.2	37.0	44.5	42.5	43.0
	W	66.3	61.4	47.7	56.5	67.1	75.4
	WL	63.9	54.9	59.4	84.1	60.0	70.8
	C	43.5	22.2	31.6	55.0	53.3	47.0

* W = Chlorinated water
L = Litter change
WL = Chlorinated water and litter change
C = Control

Table 9-7. Average Body Weights (lbs) of Birds on Different Diet and Litter Treatments

Diet	Treatment*	Initial	Final	Change
1	L	4.21	3.90	- .31
	C	4.00	4.60	+ .60
2	L	4.10	4.05	- .05
	C	3.92	4.03	- .11
3	L	4.02	3.85	- .17
	C	4.05	4.26	+ .21
4	L	4.02	3.79	- .23
	C	3.85	4.00	+ .15

* L = litter change
C = Built-up litter

Table 9-8. Analysis of Variance of Final Body Weights of Birds on Different Treatments

Source of variation	Degrees of freedom	Sum of square	Mean square	F ratio	F .01
Total	85	25.79			
Treatment	7	7.28	1.4	.59*	2.95
Error	78	18.51	.24		

* Since F ratio was not significant both at .01 and .05 level of probability, the further analysis of cell totals was not performed.

Diet	1	2	3	4
Average value	4.28	3.9	4.2	3.77

Treatments	C	L
Average value	4.03	3.9

Means underscored by the same line are not significant at 0.01 or 0.05 level of probability.

Table 9-9. Percent Fertile and Percent Hatch of Fertile Eggs Set From Birds Given Litter Change and Built-up Litter Treatments

Diet	Treatment	Jan. 21, 1963			March 11, 1963			Apr. 8, 1963	
		% Fert.	% Hatch.		% Fert.	% Hatch		% Fert.	% Hatch
1	Control	(86) 87.2	90.7	(45)	96.0	91.0	(86)	94.2	88.8
	Litter change	(103) 96.0	95.9	(76)	96.0	94.0	(74)	94.6	94.2
2	Control	(46) 95.0	97.7	(8)	--	--	(55)	92.8	96.1
	Litter change	(57) 82.4	93.6	(51)	75.0	94.0		--	--
3	Control	(42) 2.4	100.0	(33)	79.0	88.0	(54)	79.6	95.3
	Litter change	(46) 69.6	78.1	(7)	71.0	60.0	(28)	89.3	88.0
4	Control	(19) 94.7	100.0	(40)	80.0	88.8	(45)	88.9	95.0
	Litter change	(41) 9.7	75.0	(34)	73.0	92.0	(54)	70.4	81.6

The number in parenthesis indicates the number of eggs set at different dates.

Summary

Fertility:

Diets	1	2	3	4
Av. value (%)	94.4	70.7	69.0	69.5

Treatments:	Control	Litter change
Av. value (%)	76.0	75.4

Hatchability:

Diets	1	2	3	4
Av. value (%)	92.4	79.2	84.9	88.6

Treatments	Control	Litter change
	85.8	86.7

Table 9-10. Summary of Production and Quality of Eggs From Hens Under Different Treatments

Diet	Treatment	Percent prod. hen/day*	Size, quality and shell			
			No. eggs	Av. wt. (gms)	Haugh Score	Shell thickness (.001 inch)
1	Control	75.5 (A)	80	55.2	74.8	13.7
	Litter change	72.7 (A)	75	53.3	74.5	13.7
	Chlorinated water	66.0 (A)	55	56.7	74.9	13.9
	Litter change and chlorination of water	70.0 (A)	65	54.2	74.5	13.4

* Means having same capital letter are not significantly different ($P < .01$).

Table 9

Diet	
1	
2	
3	
4	

Treat

Diet

* E

Table 9-11. Effect of Different Diets and Treatments on Egg Weights*

Diet	No. eggs	Control	Litter change	Chlorine in drinking water	Litter change change and chlorine in water
		(gms)	(gms)	(gms)	(gms)
1	160	55.2	53.3	56.7	54.2
2	150	54.8	55.1	53.3	53.0
3	180	48.2	52.4	55.2	55.9
4	140	51.5	56.1	56.0	56.9
Treatments		52.4	54.2	55.3	55.0
Diets		54.8	54.0	52.9	55.1

* Expressed in grams

Table 9-12. Summary of Results Obtained From Agglutination Test Using Salmonella antigen O Group D*

Diet	Total birds tested	Treatments*				Reaction (+ or -)
		Litter	Chlorine water	Both	Control	
1	8	x				-
2	6	x				-
3	5	x				-
4	1	x				1
1	3		x			-
2	3		x			-
3	5		x			-
4	4		x			-
1	6			x		-
2	6			x		-
3	8			x		-
4	5			x		-
1	5				x	-
2	1				x	-
3	9				x	-
4	5				x	-

* Available from Difco Laboratories, Detroit, Michigan
** X represents treatment

Table 9-13. Summary of Results of Agglutination Test Using *E. coli* Antigens*

Diet	No. serum samples tested	Management treatment	Reaction with antigen H	Reaction with antigen O**
1	8	Chlorine water + litter change	1	-
	8	Control	1	-

* Supplied by Dr. D. E. Schoenhard and his staff, Dept. of Microbiology and Public Health.
Test tube consisted of 0.5 ml of serum and 0.5 ml of antigen.
Test tubes were incubated for 12 hours and refrigerated afterwards for 4 hours.

** Prepared from H antigen by boiling for 1/2 hour in a water bath, centrifuged, washed bacterial sediment with normal saline. Reconstituted with same amount of normal saline.

Table 9-14. Differential Leukocytic Counts (percentages) From Birds * Kept Under Different Management Treatments

Treatment	Lymphocyte	Monocyte	Heterophils	Eosinophils	Basophils
Control	70.25	2.0	23.1	1.87	2.75
Litter change	68.10	2.5	25.2	1.90	2.25
Litter change and chlorine in water	67.10	2.0	27.7	1.37	1.75

* Birds fed diet 1

Table 9-15. Blood Coagulation Time for Birds Kept on Different Diet and Management Treatments

Diet	No. of sample	Treatment	Average time (seconds)
1	6	Chlorinated water and litter change	56
	6	Control	70
3	5	Litter change	66
	5	Chlorinated water	80

Table 9-16. Summary of Blood Glucose Levels in Birds Receiving Different Management Treatments

Diet	Treatment	No. samples examined	Av. blood glucose (mg %)
1	Control	22	226 (A)
	Litter change	22	222 (A)
	Litter change and chlorinated water	22	242 (B)

Means not indicated by the same capital latter are significantly different ($P < .01$)

Table 9-17. Analysis of Variance of Blood Sugar Levels Among Birds Kept on Different Management Treatments

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio	F .01
Total	66	19,134			
Treatments	2	4,991	2,496	11.3	4.98
Error	64	14,143	221		

Treatments	C	L	LW
Average values	226	222	242

Means not underscored by the same line are significantly different ($P < .01$).

Table 9-18. Summary of Blood Urea Nitrogen Levels in Birds* Receiving Different Management Treatments

Treatment	No. of samples	Av. blood urea nitrogen (mg %)
Control	22	1.89
Litter change	22	1.86
Litter change and chlorination of water	22	1.61

* Birds fed diet 1

Table 9-19. Summary of Plasma Protein Levels in Birds Receiving Different Management Treatments

Diet	Treatment	No. of samples	Av. plasma protein gms/ 100 ml of blood
1	Control	22	6.75
	Litter change	22	7.13
	Litter change and chlorinated water	22	7.52

EXPERIMENT X

The Effect of Various Diets and Litter Treatments Upon The Incidence of Mortality in Laying Hens.

All the experimental procedures were the same as described for Experiment IX with the following additions or modifications. Six-hundred 24-week-old, commercially-hatched White Leghorn-type hens were randomized into six groups, based on weight and egg production, and fed the control or experimental egg-laying diets. The dietary treatments consisted of soybean oil meal and raw ground soybeans with and without supplementation of methionine, sodium carbonate, detergent and antibiotic (Pro-strep, a combination of one part procaine penicillin and three parts streptomycin). There were four replicate pens on each diet and pens having odd and even numbers were given different litter treatments. The compositions of the basal rations and experimental plans are shown in Tables 10-1 and 10-2. Different litter treatments employed were as follows:

A. Frequent change of litter. From pens having odd numbers, old shavings were removed and replaced by a ½-inch thick layer of new shavings once a week.

B. Built-up litter: At the start of the experiment, pens having even numbers were provided 8-10 inches of new shavings. At intervals of eight weeks, some fresh litter was added and stirred lightly into old litter.

Chlorination of drinking water as used in previous Experiment (IX) was not attempted.

For control of pickouts, specks were used.

Two replicate pens from diet 1, with different litter treatments were selected for bacteriological, coccidiosis and moisture determination studies.

Samples of litter and droppings were examined to determine whether any oocysts present were sporulating. An attempt was made to differentiate pathogenic species of coccidia, as they existed in these samples. For this purpose, the sugar flotation concentration method (Morgan and Hawkins, 1953) was used as follows: The collected samples were mixed with water, allowed to stand for one-half hour, mixed, strained through a layer of cheesecloth, and poured into two 15 ml, round-bottom centrifuge tubes (approximately one-quarter full). Saturated sugar solution was added until the tubes were filled. These tubes were then centrifuged for one minute at approximately 1,000 r.p.m. Using a beaded glass rod, the material from the surface solution was transferred to a glass slide and covered with a coverslip. An examination of this material was made using a compound microscope (100X), for the presence of oocysts.

The oocyst counts from litter and droppings were made as reported by Young (1929) with the following additions or modifications: five grams of fresh droppings were collected and carefully weighed into a 100-ml Erlenmeyer flask. Forty-five milliliters of distilled water were added and the contents of the flask shaken vigorously until the total mass became thoroughly emulsified. Ten milliliters of the emulsion which contained one gram of the sample were transferred to a round-bottomed 15 ml centrifuge tube. An electric centrifuge at a low speed of 1,000 r.p.m. for a period of one minute was used to throw down the oocysts to the bottom of the tube, and the supernatant fluid was decanted. To the residue, a sufficient amount of saturated sugar solution was added to bring the level of the liquid to the upper edge of the centrifuge tube. A thick coverslip (No. 3) was placed over it in such a way that no air bubbles appeared below the coverslip. A low centrifuge speed of about 1,000 r.p.m. continued for about one minute to force the oocysts to the top of the tube where they were collected on the undersurface of the coverslip. The coverslips were removed and mounted on a slide. The oocyst counts were

made using the low power lens of the microscope. This process was repeated until no oocysts were found in the specimen preparation. The total of the amounts obtained from individual coverslips gave the number of oocysts in one gram of the sample. Dead birds from these pens were especiallychecked for coccidiosis. For this purpose, smears were made of intestinal scrapings to detect presence of oocysts.

To pursue further any infectiveness of different litter treatments, a study was undertaken with baby chicks as follows: 200 one-day-old, incubator hatched White Leghorn, straight-run chicks were distributed into two brooder pens each with a different source of floor litter. Used shavings from two replicate pens receiving different litter treatments were spread on the floor of two brooder pens. The remaining two replicates continued as originally planned. This experiment was continued for a period of 21 days.

During this period the droppings of these young chicks were closely examined for blood or mucous discharge. At regular intervals, feces from these chicks were collected and examined for presence of oocysts. At the end of the first and second week of the experiment, 10 birds per treatment were sacrificed and intestines including ceca were examined for coccidiosis. A chick starter containing no antibiotic or coccidiostat (Appendix Table 5) was fed ad libitum. Each group was given a weighed quantity of feed which was reweighed at the end of the experimental period. All birds were regularly fed and watered every day. When birds died, a necropsy was performed to ascertain whether or not coccidia could have caused the death. Group weights were taken as soon as the birds were put on experiment and at the end of the second week. Individual weights were recorded at the end of the experiment. At this time, 25 birds per group were removed to an avian necropsy room and sacrificed and their intestines including ceca checked for coccidiosis lesions. Any lesions were further checked by either of the following methods depending on their nature.

1. Intestinal epithelium was scraped and smear preparations were examined using a low power of microscope.
2. Intestines including ceca were removed and cut lengthwise. The epithelium was scraped and contents were drained into a 50-ml Erlenmeyer flask which was one-half filled with tap water. This material was placed in a Waring blender and subjected to its action for 1.5 minutes. The material from the blender jar was poured into two 15-ml centrifuge tubes and centrifuged at approximately 1,000 r.p.m. for two minutes. The supernatant was poured off and the tubes refilled with tap water. This procedure was repeated until the supernatant was clear. The supernatant was then removed and saturated sugar solution was added. The rest of the procedure was the same as described on page 68.

To determine the moisture content, samples of both types of litter were collected and twenty-gram samples were weighed into well-cleaned and dry beakers. These were dried completely in an oven at 100° C. for 48 hours and reweighed.

For bacteriological studies, ten grams of the well-mixed representative litters were placed in a sterile Erlenmeyer flask and 90 grams of sterile distilled water added. This material was then shaken vigorously (about 150 times) and filtered through sterile cheesecloth into another sterile container. Filtrate was made into a number of serial dilutions before plating so as to have count ranges from 30 to 300 colonies per plate. The method of preparation of nutrient media used in this study has already been described under Experiment I on page 23.

The criteria used for evaluation of different litter treatments were rate of mortality, relative infectiveness of the litter to chicks, rate of egg production, egg size, fertility and hatchability of eggs from the hens

receiving different management treatments. Initial and final body weights of the experimental birds were also determined. During each month one week's eggs were broken out in order to determine differences in the number of bloodspots, weight, Haugh scores and shell thickness of eggs produced by the experimental birds kept on different management treatments and fed diets having different soybean variables.

Various litter management practices under different diet treatments had statistically nonsignificant ($P < .05$) effect on egg production and mortality rate. Egg production for birds kept on litter change treatment was higher than for those kept on built-up litter. There was significantly ($P < .05$) less mortality among the birds receiving diets 10-1, 10-2 and 10-3. These results are summarized in Tables 10-3, 10-5 and 10-6. Egg production in these birds for January was higher than that for February and March (Table 10-7a and 10-7b). Death rate for a period of four months (December to March) was relatively higher than during October and November when these birds were not in production (Table 10-5A and 10-5B).

Leukosis, ruptured yolk and peritonitis were the primary causes of death. Other isolated cases which occurred included sinusitis, nephritis, synovitis, CRD, fatty degeneration of liver, lymphoid mesenteric tumor and impacted oviduct. Once again, the incidence of the visceral form of leukosis was predominantly higher than its other forms. Infections associated with the reproductive system were more common than others. Mortality data are also shown in Tables 10-3 and 10-5 and the analysis of variance in Table 10-4.

The egg production records were maintained for five months, with trapping for 110 days during the period (November through March). Variability in diets had a significant effect ($P < .01$) on egg production. During this five-month period of time, egg production was significantly depressed ($P < .01$) by ration 10-2. The soybean oil meal containing ration (Diet 10-1) produced the best results but this rate of production was not significantly better

from that produced by the experimental diet (10-4) containing raw soybeans with supplemental methionine and antibiotic, Pro-strep. The egg production on diet 10-3 which contained 0.16 percent methionine plus 0.1 percent of sodium carbonate was not statistically different from that produced by diets 10-1, 10-4 and 10-6. These results are shown in Tables 10-6, 10-7 and the analysis of variance are given in Table 10-8. The percentage of bloodspots in eggs examined was very low and the inclusion of various soybean variables in the ration under different management practices did not produce an increase in their incidence. There were no differences due to diets and/or management treatments in average egg weight, Haugh scores or egg shell thickness (Table 10-9). The weight of the birds kept on litter change treatment was not statistically different from the weight of the birds housed on the built-up litter floor. The birds on each diet gained weight. The birds receiving diet 10-4 gained more than any other group. The differences in final body weights reflected to an extent the adequacy of the diets being fed. The results are summarized in Table 10-10.

Eggs laid during a five-day period in December, January and February were incubated and the percentage of fertile eggs and the percentage hatched of fertile eggs determined. Satisfactory fertility and hatchability were maintained on most of the experimental rations. However, the fertility and hatchability produced by diet 10-1 was very low. This depression was due mainly to one replicate pen of this diet. No explanation seems apparent for these results. Perhaps the males present in these pens lacked fertility (Table 10-11).

Total bacterial counts per gram of litter did not differ significantly among the different treatments (Tables 10-12, 10-13). However, the moisture content of the litter changed frequently was higher than that of built-up litter. The third day after changing litter, the moisture content was as high

as that of built-up litter. In pens where litter was changed, the floor litter appeared damp and in poor condition throughout the winter months. In the adjacent pens with built-up floor litter, the litter was in good condition. Of course, temperature at the top of the litter or down at the floor did not vary in pens having different litter treatments. These results are shown in Table 10-14.

Various litter treatments had similar effects on oocyst contents of litter and droppings. However, slightly lower counts were observed in droppings of birds housed on built-up litter. E. Acervulina was most widespread specie of coccidia found in a greater number of the samples of litter and droppings examined in both litter treatments. Ascarid ova were seen only in two samples examined from built-up litter floor pens. These results are shown in Tables 10-15 and 10-16.

There were non-statistical ($P < .05$) differences in feed conversion and gain in body weights of chicks reared on different types of litter treatment. Greater gains in body weights and better feed efficiency resulted with chicks reared on litter transferred from pens where it was changed frequently than in chicks raised on litter from built-up litter pens. These results are shown in Table 10-17 and the analysis of variance for these data are given in Table 10-18.

During the first weeks of the experiment, when birds were sacrificed, the number of infected birds and the presence of hemorrhagic ceca varied with the group. At the end of the experiment, the sacrificed birds failed to show any evidence of coccidiosis infection, except for a few oocysts present in the cecal contents (Table 10-19).

During the first three days of the experiment, nine chicks were found dead in both experimental groups. Autopsy on these birds showed absence of hemorrhages in ceca, as well as absence of merozoites in cecal and

intestinal scrappings. Pathological conditions observed in these birds were air sacculitis, peritonits and other nonspecific conditions.

Table 10-1. Experimental Plan and Composition of Original Six Experimental Rations for Experiment X

Ration number	Pen number*	Composition**
10-1	9, 18, 37, 46	Positive control ration using 44% protein soybean meal
10-2	4, 11, 36, 43	Corn, raw ground soybeans plus .16% methionine
10-3	10, 17, 38, 45	As 10-2 plus 0.1% sodium carbonate
10-4	8, 15, 40, 47	As 10-2 plus Pro-strep (50 gms/ton)
10-5	5, 14, 33, 42	As 10-2 plus .05% detergent (Solar F-221, Swift & Co.)
10-6	7, 16, 39,48	As 10-5 plus Pro-strep (50 gms/ton)

* Pens having odd numbers were given litter change treatment.

** See Table 10-2 for the exact amounts of ingredients used.

Table 10-2. Composition of Layer-breeder Rations for Experiment X

Ingredients	Percent of ration No. 10-1	10-2	10-3	10-4	10-5	10-6
Corn, ground yellow	59.90	59.90	59.90	59.90	59.90	59.90
Raw soybeans, ground	--	21.40	21.40	21.40	21.40	21.40
Soybean oil meal, 44% prot.	21.40	--	--	--	--	--
Fish meal and solubles, 60% prot.	2.0	2.0	2.0	2.0	2.0	2.0
Alfalfa leaf meal, 20% prot.	2.50	2.50	2.50	2.50	2.50	2.50
Limestone, ground	6.0	6.0	6.0	6.0	6.0	6.0
Dicalcium phosphate, ground	1.20	1.20	1.20	1.20	1.20	1.20
Salt, iodized	.25	.25	.25	.25	.25	.25
Fat, animal	4.50	4.50	4.50	4.50	4.50	4.50
Vitamin-trace mineral mix*	.25	.25	.25	.25	.25	.25
DL-methionine	--	.16	.16	.16	.16	.16
Sodium carbonate	--	--	0.1	--	--	--
Detergent (Solar F-221)	--	--	--	--	.05	.05
Antibiotic**	--	--	--	+	--	+

* Nopco Chemical Co., Harrison, New Jersey
** Pro-strep 50 gms/ton
Calculated analysis for ration:

Protein	%	17.67	Glycine	%	0.90
Fat	%	7.27	Methionine	%	0.35
Fiber	%	3.42	Cystine	%	0.25
Calcium	%	3.00	Lysine	%	0.90
Phosphorus	%	0.68	Tryptophan	%	0.19
Arginine	%	1.02			

Vitamins:		Amt/lb	Vitamins		Amt/lb
B	IU	4021	Niacin	mg	19.89
D_3 (added)	I.C.U.	750	Pantothenic acid	mg	5.79
E	mg	5.04	Choline	mg	537.00
B_{12}	mcg	6.26	Folic acid	mg	0.23
Riboflavin	mg	2.37	Prod. energy	Cal/lb	983.

Table 10-3. Incidence of Mortality Under Various Diets and Management Treatments

Diet	Treatment**	Mortality %*	Postmortem findings***											
			ST	CRD	RUY-P	IMO	VL	NL	P	LMT	H	SY	N	O
1	C	6			1			1						1
	B	4			1		1							
2	C	4											1	1
	B						1							1
3	C	4									1		1	
	B	2							1					
4	C	10			1		3						1	
	B	12	1	1	1	1	1			1				
5	C	12					3				1	1		1
	B	10			2		2		1					
6	C	10			1		3		1					
	B	4					1						1	
	Total		1	1	7	1	15	1	3	1	2	1	4	4

*Significant at 0.05

** C = Litter change
B = Built-up litter

*** Key to findings:

ST = sinusitis and tracheitis
CRD = Chronic respiratory disease
RUY-P = Ruptured yolk and/or peritonitis
IMO = Impacted oviduct
VL = Visceral lymphomatosis
NL = Neuro-lymphomatosis
P = Pickouts
LMT = Lymphoid mesenteric tumor
H = Hepatitis
N = Nephritis
SY = Synovitis
O = Other nonspecific cases

Table 10-4. Analysis of Variance of Mortality Data for Birds in Experiment X

Source	Sum of squares	Degrees of freedom	Mean square	F ratio	F .05
Treatments	14	1	14.0	3.9	6.61
Diets	194	5	38.8	10.8*	5.05
Error	18	5	3.6		
Total	226	11			

Standard error of the mean = 1.33

* Significant at P < .05

Treatments:	C	B
Means	7.66	6.00

Diets:	4	5	6	1	2	3
Means	22	22	14	10	8	6

Means not underscored by the same line are significantly different at 0.05 level of probability.

Table 10-5A. Summary of Postmortem Findings Showing Incidence of Leukosis and Reproductive Disorders Under Various Diets and Management Treatments

Diet	Treatment*	Leukosis including other tumors	Diseases associated with reproductive system	Others
1	C	1	1	1
	B	1	1	-
2	C	-	-	2
	B	1	-	1
3	C	-	-	2
	B	-	1	-
4	C	3	1	1
	B	2	2	2
5	C	3	-	3
	B	2	3	-
6	C	3	2	-
	B	1	-	1
	Total	17	11	13

* C = Litter change
B = Built-up litter

Table 10-5B. Monthly Incidence of Mortality in Various Experimental Pens Under Different Diets and Treatments

Diet	Pen no.*	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Monthly av. mortality (%)
1	9	-	-	-	-	-	1	.67
	37	-	-	-	1	1	-	1.33
	18	-	-	-	-	-	-	0
	46	-	-	-	-	1	1	1.33
2	11	1	-	-	-	-	-	.67
	43	-	1	-	-	-	-	.67
	4	-	-	-	-	-	-	0
	36	-	-	-	-	-	2	1.33
3	17	-	-	-	-	-	1	.67
	45	-	-	-	-	1	-	.67
	10	-	-	1	-	-	-	.67
	38	-	-	-	-	-	-	0
4	15	1	-	-	1	-	1	2.0
	47	-	-	-	-	1	1	1.35
	8	-	-	-	1	-	-	.67
	40	-	-	2	2	1	-	3.33
5	5	-	-	-	2	2	1	3.33
	33	-	-	-	-	-	1	.67
	14	-	-	-	-	1	-	.67
	42	1	1	1	-	1	-	2.67
6	7	-	-	2	-	1	-	2.0
	39	-	-	-	-	1	1	1.33
	16	-	-	-	-	1	-	.67
	48	-	-	-	-	-	1	.67

* Odd number pens were given litter change treatment.
Each experimental pen had 25 birds at the start.

Table 10-6. Summary of Egg Production and Mortality (percentage) With Respect to Diet and Management Practices

Diet	Treatment*	Av. egg production (Nov. - Mar.) (%)	Mortality (%)
1	C	57.4	6
	B	58.2	4
2	C	45.6	4
	B	47.5	4
3	C	56.6	4
	B	50.9	2
4	C	56.5	10
	B	57.2	12
5	C	51.4	12
	B	56.4	10
6	C	58.7	10
	B	53.8	4

* C = change of litter
B = Built-up litter

Diets	1	4	6	3	5	2
Av. prod. (%)	57.8	56.8	56.7	53.8	53.7	46.6

Treatments:	C	B
Av. prod. (%)	54.4	54.0

Means not underscored by the same line are significantly ($P < .01$)

Table 10-7A. Percent Egg Production Under Various Diets and Management Treatments Computed on the Basis of Total Number of Live Birds Present at the Beginning of each month

Diet	Treatment*	Nov.	Dec.	Jan.	Feb.	March	Average production Dec.-March
1	C	12.5	66.5	71.0	67.6	62.6	66.9
	B	9.0	67.8	68.0	70.0	71.3	69.3
2	C	7.0	56.4	57.8	47.1	55.2	54.1
	B	9.2	55.8	57.2	52.9	59.2	56.3
3	C	11.9	64.7	68.3	64.0	69.4	66.6
	B	15.1	63.0	59.3	56.4	57.6	59.0
4	C	12.5	64.9	69.8	61.6	70.6	66.7
	B	12.9	71.0	67.7	60.7	66.4	66.5
5	C	11.9	57.2	61.1	62.2	61.7	60.6
	B	8.5	61.9	69.9	64.2	68.3	66.0
6	C	11.5	71.1	68.2	63.1	71.8	68.6
	B	10.0	65.3	61.4	60.8	65.1	63.2

* C = litter change
B = built-up litter

Table 10-7B. Percent Egg Production Under Various Diets and Management Treatments Computed on Basis of Total Number of Birds in Production Each Month

Diet	Treatment*	Nov.	Dec.	Jan.	Feb.	March
1	C	22.9	82.1	79.0	78.0	75.5
	B	16.2	88.8	80.9	83.2	87.7
2	C	19.0	85.1	78.2	68.8	79.5
	B	12.3	76.1	64.7	65.5	71.1
3	C	20.8	96.5	72.9	70.4	75.5
	B	16.2	82.9	72.8	71.0	68.8
4	C	24.0	89.9	83.0	72.4	79.4
	B	22.4	93.8	78.2	75.5	81.1
5	C	15.9	76.1	68.2	68.3	75.4
	B	16.9	79.9	76.0	66.8	75.1
6	C	19.9	84.1	76.6	73.0	80.8
	B	17.7	85.5	72.4	77.0	76.3

* C = Change of litter
B = Built-up litter

Table 10-8. Analysis of Variance of Egg Production Data With Respect to Diets and Management Treatments

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F .01
Diets (D)	5	4673	934.6	7.3*	3.06
Treatment (T)	1	492	492	3.84	6.76
Interaction (D X T)	5	823	164.6	1.28	
Subtotal	11	5988			
Error	408	52239	128.0		
Total	419	58227			

* Significant both at .01 level of probability

Diets	2	5	3	6	4	1
Means	46.6	53.7	53.8	56.7	56.8	57.8

Treatments	C	B
Means	54.4	54.0

Means not underscored by the same line are significantly different ($P < .01$)

Table 10-9. Summary Showing Quality of Eggs from Hens Receiving Different Experimental Diets and Management Treatments

Diet	Composition	Treatment*	Size, quality and shell: No. eggs	Av. wt. (gms)	Albumen height	Haugh score	Shell thickness (.001 inch)	Number bloodspots
10-1	Positive control, corn, soybean meal	C	48	55.5	6.1	78.7	14.6	1
		B	38	56.6	5.5	74.2	15.9	0
10-2	Raw soybeans (ground), corn, plus .16% methionine	C	48	55.8	6.2	79.5	15.2	1
		B	37	55.8	6.0	78.2	15.0	0
10-3	Diet 10-2 + 0.1% Na_2CO_3	C	47	54.0	6.1	79.5	14.7	1
		B	46	56.1	6.0	77.5	15.2	2
10-4	Diet 10-2 + 50 gms/ton Pro-strep	C	48	56.9	6.5	81.5	15.2	1
		B	48	56.1	6.4	81.0	15.3	2
10-5	Diet 10-2 + .05% detergent (Solar F-221, Swift)	C	49	55.9	6.2	79.7	15.0	2
		B	49	54.5	5.8	77.5	15.4	1
10-6	Diet 10-5 + 50 gms/ton Pro-strep	C	49	56.3	6.5	81.7	15.2	3
		B	42	56.5	5.9	77.2	14.8	1

* C = Change of litter
B = Built-up litter

Table 10-10. Average Body Weights (lbs.) of Birds Kept on Different Diets and Litter Treatments During Experiment X

Diet	Treatment*	Initial	Final	Change
1	C	2.81	3.99	+ 1.18
	B	2.85	4.08	+ 1.23
2	C	2.94	3.88	+ .94
	B	2.86	3.73	+ .87
3	C	2.98	3.93	+ .95
	B	2.82	3.90	+ 1.08
4	C	2.98	4.22	+ 1.24
	B	2.91	4.08	+ 1.17
5	C	2.78	3.76	+ .98
	B	2.86	3.90	+ 1.04
6	C	2.84	4.08	+ 1.24
	B	2.91	4.11	+ 1.20

* B = Built-up litter
  C = Litter change

Table 10-11. Percent Fertile and Percent Hatch of Fertile Eggs Set From Birds on Different Litter Management Treatments in Experiment X

Diet	Treatment*	Dec. 16, 1963			Jan. 13, 1964			Feb. 10, 1964		
			% Fert.	% Hatch		% Fert.	% Hatch		% Fert.	% Hatch
1	B	(155)	45.9	39.8	(155)	49.0	43.3	(53)	48.5	44.0
	C	(125)	95.2	79.0	(144)	96.3	94.4	(66)	98.0	95.0
2	B	(102)	93.5	80.1	(114)	77.0	89.2	(37)	89.0	87.0
	C	(101)	86.1	82.6	(111)	93.5	91.4	(64)	95.0	96.0
3	B	(134)	93.4	84.1	(132)	79.6	95.2	(78)	94.5	98.0
	C	(109)	88.6	86.3	(61)	95.0	96.4	(63)	94.0	98.0
4	B	(127)	82.2	88.7	(138)	92.1	93.6	(56)	94.0	92.5
	C	(149)	94.6	75.2	(136)	94.0	80.7	(37)	82.5	91.0
5	B	(120)	94.8	78.3	(126)	99.9	96.7	(77)	93.0	96.0
	C	(112)	92.5	77.6	(133)	95.5	89.2	(52)	92.0	93.5
6	B	(137)	92.0	90.5	(126)	92.2	95.1	(64)	93.0	91.0
	C	(119)	64.6	90.3	(134)	96.7	85.9	(66)	92.5	94.0

The number in parenthesis indicates the number of eggs set at different dates

* C = Change of litter
  B = Built-up litter

Summary

Fertility:	Diets	1	2	3	4	5	6
	Av. values (%)	72.1	89.0	90.8	89.8	94.6	88.5
Treatment:		B		C			
	Av. value (%)	83.5		91.4			
Hatchability:	Diets	1	2	3	4	5	6
	Av. values (%)	65.9	87.7	93.0	86.9	88.5	91.1

Table 10-12. Bacterial Counts From Litter Changed Frequently

Fresh litter Added (days)	Litter samples obtained from	Bacterial colonies/ gm of litter (thousandths)
1	Center of floor	3,120
1	Center of floor	66,000
1	Near water fountain	14,000
1	Near water fountain	38,000
1	Floor under laying nests	158,000
2	Center of floor	384,000
2	Near water fountains	304,000
2	Center of floor	84,000
2	Floor under laying nests	1,024,000
2	Floor under laying nests	402,000
2	Near water fountains	734,000
3	Floor under laying nests	176,000
3	Center of floor	434,000
3	Near water fountains	202,000
3	Floor under laying nests	596,000
3	Near water fountains	135,000
3	Near entrance	382,000
4	Center of floor	440,000
4	Near water fountains	172,000
4	Under laying nests	434,000
4	Floor	1,426,000
5	Near water fountains	696,000
5	Near entrance door	1,978,000
5	Center of floor	220,000
5	Floor under laying nests	230,000
5	Near water fountains	1,724,000
5	Floor adjacent to dropping pit	140,000
7	Near entrance door	888,000
7	Near water fountains	4,150,000
7	Floor under laying nests	652,000
7	Floor adjacent to dropping pit	172,000
7	Center of floor	1,278,000
7	Floor under laying nest	725,000
7	Near entrance door	1,525,000
7	Near water fountains	725,000

Table 10-13. Bacterial Counts From Litter Unchanged During Experiment X

Litter samples obtained from					
Center of floor	Near water fountains	Floor under laying nests	Near entrance door	Floor adjacent to the dropping pits	Bacterial colonies/gm of litter (thousandths)
	x				324,000
				x	1,384,000
		x			1,840,000
			x		170,000
x					576,000
x					4,600
		x			13,600
	x				126,000
x					36,000
		x			748,000
			x		69,200
x					125,000
		x			1,354,000
				x	132,000
	x				78,000
		x			1,400,000
x					1,248,000
		x			131,000
			x		376,000
x					145,000
				x	125,000
x					1,850,000
		x			720,000
	x				680,000
			x		172,000,000
				x	85,000
x					180,000
	x				725,000

Table 10-14. Moisture Content of Litter Used for Housing Birds in Experiment X

Interval after cleaning (days)	Number samples examined	Average moisture content (%)
2	5	23
3	5	29
4	5	41
5	9	44
Built-up litter	10	30.5

Table 10-15. Summary of Examination of Litter and Droppings for Coccidial Oocysts and Ova From *Ascaridia lineata* and *Heterakis gallinae**

Sample	Treatment	Number samples examined	Coccidial oocysts	E. nectrix E. tenella	E. Acervulina E. Maxima & other species coccida	Heterakis gallinae	Ascaridia lineata
Layer of litter	3rd day after changing litter	3	1	1	0	0	0
Litter mixed w/droppings	5th day after changing litter	5	3**	1	2	0	0
Droppings	5th day after changing litter	7	2	2	2	0	0
Layer of litter	Built-up litter	3	1	1	1	0	0
Half-inch deep layer of litter	Built-up litter	2	0	0	0	0	0
Litter mixed w/droppings	Built-up litter	4	2**	0	2	0	1
Droppings	Built-up litter	6	2	1	2	0	1

* For oocyst counts see Table 10-16
** Sporulated oocysts present

Table 10-16. Coccidial Oocyst Counts from Litter and Droppings

Sample	Treatment	No. of oocysts gram of sample
Litter mixed with droppings	5th day after changing litter	67
Litter mixed with droppings	5th day after changing litter	47*
Fresh droppings	5th day after changing litter	212
Fresh droppings	5th day after changing litter	144
Litter mixed with droppings	Built-up litter	42*
Litter mixed with droppings	Built-up litter	37*
Fresh droppings	Built-up litter	92
Fresh droppings	Built-up litter	86

* Sporulated oocysts present

Table 10-17. Feed Intake, Feed Efficiency, Gain in Weight and Mortality Rate of Three-week-old White Leghorn Chicks Used in Experiment X for Determination of Relative Infectiveness of Litter Under Different Management Treatments

Treatment	Source of litter	Av. wt./bird (gms)	Feed consumed/bird (gms)	Feed/gain	Mortality (%)
B	Upper layer of "built-up" litter used for housing laying hens in Experiment X	183.1	445	3.11	6
C	From pens where litter was changed frequently during Experiment X	187.2	466	2.98	3

Treatments	B	C
Av. wt./bird	183.1	187.2

Means underscored by the same line are nonsignificantly different at 0.05 level of probability

Table 10-18. Analysis of Variance of Three-week Body Weights of Chicks Used in Experiment X for Determining Relative Infectiveness of Litter Under Different Litter Treatments

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F .05
Total	149	138,371			
Treatments	1	638	638	.68*	3.91
Error	148	137,733	.931		

* Nonsignificant at .05 level of probability

Treatments	B	C
Av. wt./bird	183.1	187.2

Means underscored by the same line are not significantly different ($P < .05$)

Table 10-19. Summary of Results Obtained From Chicks Sacrificed at Different Intervals for Determining Relative Infectiveness of Litter Resulting From Various Management Practices

Treatment*	Sacrificed: No. birds	Sacrificed: Interval (wk)	Enlargement of ceca	Hemorrhages or bloodspots in cecum	Coccidial oocysts in cecal contents or merozoites in the cecal or intestinal scrapings
B*	10	1	6	6	4
C*	10	1	3	2	2
B	10	2	2	2	2
C	10	2	5	3	2
B	25	3	3	-	2*
C	25	3	2	-	2*

B - Source of litter was "built-up" used for housing birds in Experiment X.

* - A few coccidial cysts were seen

C - Source of litter in this treatment was the frequently changed litter from pens cleaned weekly during Experiment X

DISCUSSION

PART I

From the review of literature and results reported herein, it is obvious that chlorine possesses bactericidal effect (Ehleres and Steel, 1927), and destroys many bacteria of the pathogenic and coli groups. Disinfection by chlorine (Anon, 1949) depends on the concentration of residual chlorine, and thus the amount of organic impurities that get in the water is important. Feed added to chlorinated water is oxidized and the nascent oxygen no longer is present. However, if the chlorinated water remains free from contamination, it would kill bacteria, especially gram-negative ones, and certain viruses if present in feed when the birds consumed it. Thus, chlorination of drinking water may prevent the transmission in chickens of bacterial and viral infections originating from food or water.

In an attempt to determine effective levels of chlorine in drinking water, filtrates from chlorinated water-feed mixtures were tested in a preliminary study. This study was conducted in the laboratory using variable levels of chlorine in water combined with a measured quantity of feed. Results of this study indicate that chlorine levels in filtrates depend upon the level of the chlorine used and presence of feed in the mixture.

Results from Experiment I showed that higher concentrations of chlorine in drinking water had a depressing effect on the intestinal bacterial flora. Possibly chlorination practiced from the time of hatching would inhibit the establishment of normal flora in baby chicks. Halbrook et al. (1951) were of the opinion that flora is established mainly through feed and water. Perhaps chlorination of the drinking water for mature birds where flora has already been established may not have similar effects.

In Experiments I and II, growth rate in control birds was significantly higher ($P < .01$) than in experimental birds supplied chlorine 250 ppm or more in drinking water. Slightly better growth resulted in control birds when compared to birds drinking chlorinated water having 100 to 250 ppm of available chlorine. Depression in growth rate increased as the levels of chlorine in drinking water increased. Whether this depression was a result of reduced feed and water intake or an effect of chlorination on intestinal microbial flora, especially E. coli and E. communior, is unknown. However, autopsy on birds did not show any pathological lesion except emaciation and empty crops. This suggests that probably it was not chlorination but starvation which resulted in their death. Of course, depression in feed and water consumption occurred as a result of higher levels of chlorination.

Results from Experiments III, IV and V conducted with young, growing chicks and increasing levels of chlorine in water show that chlorination up to 75 ppm had no depressing effect on daily water consumption although some birds consumed more water than others. Any restriction in feed and water consumption usually lowers egg production. However, the average size of the eggs does not vary. This might be the reason that some of the experimental groups of birds stopped laying altogether during the first week while others did not. These birds definitely performed better when the chlorine levels were reduced.

Severe molting was observed in all experimental birds consuming 500 ppm or higher levels of chlorination. Birds in production are very sensitive to any external stimuli and stress from thirst or insufficient feed could cause the molting observed in laying birds.

Free chlorine levels in water after 24 hours depended upon the original levels as well as upon the amount of feed added to the waterers by the beaks of the birds.

PART II

It is generally believed that the maintenance of a clean environment will reduce mortality in laying flocks (Snyder et al., 1962). Data collected in these experiments, however, did not support this view. Results from Experiment IX showed no significant differences among the various management practices as far as the mean egg production or the mortality data was concerned.

A significant difference ($P < .01$), however, occurred in egg production due to different dietary treatments. Soybean meal in the diet resulted in significantly greater egg production then did the other soy bean variables. This depression in egg production may be attributed to the experimental nature of the soybean products used. There was less mortality among birds fed the control diet and this may help explain why the performance of these chickens was better. A higher incidence of pickouts occurred among birds fed the experimental diets. Among these lots, there were ration inadequacies presently being studied. Ruptured yolks, and/or peritonitis, and leukosis ranked second and third, respectively after pickouts as causes of mortality. The visceral form of leukosis was predominantly greater than its other forms.

Higher but nonsignificant final body weights were obtained with birds fed the basal diet or kept on "built-up" litter. These results lend support to the hypothesis put forward by Halbrook et al. (1950)that chickens may obtain from the litter nutritional products such as vitamin B_{12} and riboflavin as a result of bacterial fermentation.

In general, poor fertility and hatchability were obtained by various experimental diets and management practices. The increase in percent hatchability of eggs from hens on "built-up" litter as reported by Kennard and Chamberlin (1948a, 1948b) and Sunde et al. (1951) was not found in

this experiment. No explanation is offered for these results.

Differential leukocytic count, plasma protein and blood urea nitrogen showed little variation among birds of different groups. Blood sugar levels were higher ($P < .01$) in birds given both chlorinated water and litter change treatments. Stress, stemming from diet and litter treatments may have been responsible for these increased values but, of course, these results were obtained on a relatively small number of birds. Since blood sera was not agglutinated with Salmonella and E. coli, specific strain antigens, it is likely that these birds had little exposure to these specific infections. Certain inhibitors might be responsible for an anticoagulant activity in blood of chickens provided raw soybeans.

In Experiment X, a ;significant difference ($P < .01$) resulted in egg production from various diet treatments. The .16 percent level of methionine appeared to be of little or no benefit when added singly or in combination appeared to be of little or no benefit when added singly or in combination with .05 percent detergent to a ration containing raw soybeans. These results are, however, in contrast to that already reported by Yates (1963) who found that .16 percent level of methionine when added in combination with .05 percent of surface-active agent to the ration containing raw soybeans significantly improved egg production. There were differences in basal rations used and moreover long-term effects of this diet is still under investigation. Antibiotic, Pro-strep, when added with supplemental .16 percent methionine to the ration containing raw soybeans (Diet 10-4) significantly improved egg production. However, when this antibiotic was added in combination with .05 percent detergent and .16 percent methionine to a ration, egg production was apparently but not significantly depressed from that produced by the diet 10-4. These results suggest that supplementation of antibiotic in rations containing raw soybeans improved egg production.

The weights of the birds housed on built-up litter were neither significantly nor apparently different from the weights of those birds kept on

pens where litter was changed frequently. This is not consistent with the results obtained previously (Experiment IX) where higher body weights were found for birds housed in built-up litter floor pens. This difference in final body weights apparently reflected to an extent the adequacy of the diet being studied. Once again, the results of hatchability suggest that built-up litter floor pens have no effect in increasing hatchability of eggs laid by birds housed in these pens.

Apparently, there were no differences in bacterial counts of litter and droppings samples examined from different litter treatments. However, isolation and characterization of various bacteria was not attempted. Perhaps bacterial flora of built-up litter may be different in nature than that of litter which is changed frequently.

There appeared to be less ($P < .05$) mortality among the birds fed the experimental diets 10-1, 10-2 and 10-3. High death rate was observed among birds fed high producing experimental diets containing raw soybeans with supplemented methionine, Pro-strep with or without detergent. Whether this occured as a result of stress of high egg production on these diets is unknown.

Various management practices had similar effects on the incidence of mortality. Death rate among birds was higher during periods of heavy egg production. During six months period of time only 41 birds died out of a total of 600; one-third of these deaths were caused by leukosis or other tumors. Individual egg records indicated that the birds that died during October, November and early December were poor layers. Perhaps this is another of nature's ways of culling weaker birds. All birds that died after December 15 were in high production. Possibly the resistance of a producing bird is lowered by stress created as a result of high egg production. This may also help to explain the increased incidence of ruptured yolks and peritonitis which may result as a consequence of some disease, hormonic

disturbance or mechanical obstructions followed by bacterial infection. Some basic work is desirable to investigate further the cause of this infection which appears predominantly in high-producing birds.

Kennard and Chamberlin (1947, 1949) have advocated the use of built-up floor litter as one of the measures for control of coccidiosis. On the other hand, Koutz (1948, 1953) found built-up floor litter a reservoir of coccidial oocysts and parasitic ova. However, the results of this study did not show differences between different litter treatments when judged on basis of coccidial oocysts and mortality data collected from baby chicks.

There were statistically nonsignificant ($P < .05$) differences in feed conversion and gain in body weights of chicks reared on different types of litter treatments. Greater gains in body weights and better feed efficiency resulted with chicks receiving litter from pen where it was changed frequently than in chicks reared on litter transferred from built-up litter pen.

During the first two weeks of the experiment, when birds were sacrificed, the number of infected birds and the presence of hemorrhagic ceca varied with the group.

This is possibly due to the nature of the coccidial organisms and the relative number of sporulated oocysts present in the litter. At the end of the experiment, the sacrificed birds failed to show any evidence of coccidiosis infection, except for a few oocysts present in cecal contents. Probably the birds were immunized resulting in discontinuation of the coccidial life cycle.

During the first three days of this experiment nine chicks were found dead in both experimental groups. Autopsy on these birds revealed absence of hemorrhages in ceca, as well as the absence of merozoites in cecal and intestinal scrappings suggesting that coccidiosis was not responsible for their death. Such results are possible because one

would anticipate mortality at the end of four to five days had infection occurred. The remaining young chicks did not develop hemorrhages and consequently showed no symptoms of coccidiosis. Probably 1 - 2-week-old birds show higher resistance to E. tenella infection (Gardiner, 1955). In addition to this, resistance of the host, breed of chickens, virulence of oocysts and whether sporulated or not, are some of the factors which may offer some explanation for these results (Herrick, 1934). These results were obtained on young chicks whether this may apply to mature birds is unknown.

Oocyst counts were relatively higher in droppings of birds housed in pens where litter was changed frequently, than in droppings of birds kept on built-up litter. Perhaps these birds had less exposure to infection, thus lacked immunity. Birds on built-up litter might have developed immunity and thus eliminated less oocysts in their feces. Of course, these results were obtained on a relatively small number of fecal samples. This variability may further be explained by the individuality of each bird as well as the stage of coccidia life cycle. Eimeria acervulina oocysts were found comparatively in large numbers of samples of litter and droppings examined from either kind of litter treatment. Perhaps this specie can withstand considerable adversity which it encounters outside the body. E. acervulina being responsible for chronic infections is possibly discharged with the feces for a longer period.

Edgar (1954, 1955) reported that sporulation of E. tenella oocysts (time involved from oocysts' passage until it becomes infective) was achieved in approximately 18 to 24 hours at 29° C. and relative humidity 60 percent. During this study, sporulated oocysts were found in samples of litter and droppings examined from pens cleaned frequently. In these pens, possibly sufficient moisture and aeration was present to sporulate coccidial oocysts. The moisture content of the litter, where it was changed frequently, was higher than that of built-up litter. The presence

of moisture in the litter not only tends to sporulate oocysts but also increases livability of nematode eggs (Koutz, 1952).

These results suggest that weekly removal of litter from poultry houses may not be advantageous judged on the basis of control of coccidiosis infection. Perhaps this might be of some significance if practiced at a much higher frequency to inhibit sporulation. Naturally, this will result in more labor and a proportional increase in expenditure. Young (1929) even has claimed that all viable oocysts whether segmented or not were potentially capable of causing infection. If this is true, then it seems unnecessary to clean the pens as often as has been recommended in the past specially for control of coccidiosis and possibly other diseases transmitted through feces.

Higher mortality observed during Experiment IX may be attributed to the following:

1. Pickouts -- This was eliminated in this experiment by successful use of specks.
2. Inadequacy of experimental diets such as inclusion of whole raw and cooked soybeans in feed formula. In this experiment, however, whole raw or cooked soybeans were not used.
3. Severe cold during winter of 1962-63 might have further lowered the resistance of the experimental birds.

The results of Experiments IX and X suggest that sanitation practices for poultry may not be advantageous over ordinary management practices with built-up floor litter when judged on the basis of mortality and mean egg production.

This may be true, because most of the diseases on a poultry farm such as this one may be nonspecific and nontransmissible in origin. The occurrence of such infections may not be prevented by better housekeeping or applying

other measures of sanitation. Infectious disease outbreaks may occur but seems to be very rare.

In a modern hen house, the average mortality per month is more than one percent (Sheppard, 1964). The results of this study showed monthly mortality of one percent. This range of mortality is probably a normal occurrence due to certain nonspecific causes and may not be prevented by sanitation practices.

Sanitary management is most likely to be useful during an outbreak of infectious diseases. Of course, during the entire period of this study, no infectious outbreak was seen on this poultry research farm.

SUMMARY AND CONCLUSIONS

PART I

Eight experiments were conducted to determine how much chlorination chickens can tolerate in the drinking water without curtailing water intake and rate of growth. Two experiments were undertaken using Leghorn-type hens to investigate the effects of chlorinated water and litter management practices on production and rate of mortality.

The following levels of chlorine in water did not affect the feed or water consumption.

50 ppm in 3-week-old chicks

75 ppm in 10-week-old chicks

25 ppm in laying hens

Lowered water intake did not reduce feed consumption or gain in body weights by chlorination at

100 to 1,000 ppm in 5-week-old chickens

50 ppm to 500 ppm in old hens

Water and feed intake as well as gain in body weights were depressed by chlorination above

100 ppm in baby chicks

1000 ppm in 5-week-old chickens

500 ppm in laying hens

Thus, the consumption of chlorinated water may depend upon the individual nature of each bird. Some birds may consume more than others. When feed and water consumption was depressed, it was reflected in body weight changes in growing chicks as well as lower egg production and molt in laying birds. The egg production in laying hens was depressed by chlorination at 50 ppm.

PART II

Frequent litter change treatments had no effect on egg production and incidence of mortality. Cannibalism, leukosis, ruptured yolks and peritonitis were the primary causes of mortality in these experiments. Other pathological conditions diagnosed were hepatitis, nephritis, sinusitis, synovitis, chronic respiratory disease and impacted oviduct which may be associated with heavy egg production. The incidence of visceral lymphomatosis was greater than that of other forms of leukosis. Infections of the reproductive system were more common than others.

Rations containing soybean oil meal produced more eggs but the rate of production of birds on these rations was neither apparently nor significantly better than that produced by the experimental diet containing raw soybeans with supplementation of methionine and antibiotic, Pro-strep. Hatchability of eggs was not improved when birds were kept on "built-up" litter.

Baby chicks brooded on litter previously used by hens kept under different management treatments did not differentiate between relative infectiveness of different litter systems.

There were no significant differences in bacterial counts of litter and droppings samples examined from different litter treatments. The moisture content of the litter was higher in the treatments receiving frequent litter change than that of "built-up" litter. Different litter treatments had similar effect on coccidial oocysts. These results suggest that chlorinated drinking water and frequent change to clean litter or both may not offer any advantages over the usual tap water and "built-up" litter, judged on the basis of mortality. This conclusion may not be applicable during an outbreak of disease. The removal of litter at such a time may be a primary step towards the control of such an outbreak.

LITERATURE CITED

Alder, B., 1934. Results of seven years of egg laying contests. Utah Agr. Exp. Sta. Bul. 248: 1.

Anderson, C. D., 1928. Report of the New York State Egg Laying Contest Nov. 1 to Oct. 23, 1927. p. 34. Issued by State Inst. of Applied Agriculture, Farmingdale, N. Y.

Anon, 1949. Editorial. Lancet 256: 1056.

Barger, E. H., Card, L. E. and Pomeroy, B. S., 1958. Diseases and Parasites of Poultry. Lea and Febiger, Philadelphia.

Barrett, F. N., 1929. Mortality as a factor in poultry production. Thesis, Univ. of Illinois, pp. 1-30.

Bezanson, J. M. and E. L. Stephenson, 1948. Factors affecting a breast blister condition in broilers. Poultry Science Ass'n. 47th Annual Meeting. p. 6.

Bird, H. R., 1947. A chick growth factor in cow manure. IV. Poultry Sci. 26: 439.

Boughton, D. C., 1939. Studies on the control of poultry coccidiosis. I. The sporulation of oocysts in various types of litter. Bul. Univ. of Georgia 39: 9.

Brackett, S. and A. Bliznick, 1952. The relative susceptibility of chickens of different ages to coccidiosis caused by Eimeria necatrix. Poultry Sci. 31: 148.

Broadfoot, D. I., B. S. Pomeroy and W. M. Smith, 1957. Effects of infective bronchitis on egg production. Jour. Amer. Vet. Med. Ass'n. 124: 128.

Brunson, C. C. and G. F. Godfrey, 1952. A fourteen-year summary of mortality in the Oklahoma Egg Laying Test. Poultry Sci. 31: 149.

Burmester, B. R., 1948. The influence of sex hormones upon the occurrence of prolapse in chickens. Poultry Sci. 27: 745.

Burmester, B. R. and R. F. Gentry, 1954. A study of possible avenues of infection with the virus of avian lymphomatosis. Proc. A.V.M.A. 311.

Burmester, B. R. and R. F. Gentry, 1955. The propagation of the virus of visceral lymphomatosis in embryonated eggs. Poultry Sci. 34: 669.

Burmester, B. R., R. F. Gentry and N. F. Waters, 1955. The presence of viruses of visceral lymphomatosis in embryonated eggs of normal appearing hens. Poultry Sci. 34: 609.

Burmester, B. R., A. B. Gross, W. G. Walter and A. K. Fontes, 1959. Pathogenecity of a viral strain (RPL-12) causing avian visceral lymphomatosis and related neoplasms. J. Nat'l. Cancer Inst. 22: 103.

Burmester, B. R. and N. F. Waters, 1955. The role of the infected egg in the transmission of visceral lymphomatosis. Poultry Sci. 34: 1415.

Burmester, B. R. and N. F. Waters, 1956. Variation in the presence of the virus of visceral lymphomatosis in the eggs of the same hen. Poultry Sci. 35: 939.

Burmester, B. R., N. F. Waters, W. R. Bryan and V. Groupe, 1960. The response of several inbred lines of White Leghorns to inoculation with the viruses of strain RPL-12 visceral lymphomatosis-erythroblastosis and of Rous sarcoma. Poultry Sci. 39: 199.

Buster, M. W., 1928. What California's cost of production studies reveal concerning poultry management. Poultry Sci. 7: 49.

Caldwell, M. J., 1957. Detecting blood coagulation defects in the clinical laboratory. Am. J. M. Tech. 23: 277.

Calnek, B. W. and P. O. Levine, 1957. Studies on experimental egg transmission of pleuro-pneumonia-like organisms in chickens. Avian Diseases 1: 208.

Card, L. E. and W. F. Kirkpatrick, 1919. Egg laying contests. Storrs Agr. Exp. Sta. Bul. 100.

Carr, J. G., 1952. The avian leukosis complex. World's Poultry Science Journal 8: 276.

Chalquest, R. P. and J. Fabricant, 1959. Survival of PPLO injected into eggs previously dipped in antibiotic solution. Avian Diseases 3: 257.

Chute, H. L. and D. C. Meara, 1957. A bibliography of avian mycosis. Maine Agr. Exp. Sta. Misc. Publ. 631.

Cole, R. K., 1949. The egg and avian leukosis. Poultry Sci. 28: 31.

Cole, R. K., 1955. Some observations on breeding fowl resistant to lymphomatosis. Poultry Sci. 34: 312.

Cole, R. K. and F. B. Hutt, 1951. Evidence that eggs do not transmit leukosis. Poultry Sci. 30: 205.

Cole, R. K. and F. B. Hutt, 1956. Parents age unrelated to leukosis in progeny. Poultry Sci. 35: 731.

Cotterill, O. J. and A. R. Winter, 1953. Some nitrogen studies of built-up litter. Poultry Sci. 32: 365.

Cottral, G. E., 1952. Endogenous viruses in the egg. The chick embryo in biological research. Ann. N. Y. Acad. Sciences 55: 221.

Cottral, G. E., B. R. Burmester and N. F. Waters, 1954a. The transmission of visceral lymphomatosis with tissue from embryonated eggs and chicks of normal parents. Poultry Sci. 33: 1174.

Cottral, G. E., B. R. Burmester and N. F. Waters, 1954b. Egg transmission of avian lymphomatosis. Poultry Sci. 33: 1174.

Couch, J. R., 1955. Cage layer fatigue. Feed Age May, 88: 55.

Crowley, T. A., A. A. Kurnick and A. R. Kemmerer, 1961. Phosphorus and laying hen performance. Poultry Sci. 40: 1391.

Darcel, C. LeO, L. Niilo, E. V. Langford and R. Connell, 1960. Mortality in White Leghorn females on a farm in Southern Alberta. Avian Dis. 4:258.

Dardiri, A. B. and O. Zaki, 1955. Mortality due to oomphalitis following air shipment of baby chicks. Poultry Sci. 34: 327.

Dawson, L. E. and J. P. Watts, 1952. Effect of nest litter on cleanliness of eggs. Poultry Sci. 31: 913.

Dudley, F. J., 1928. The death rate of three standard breeds of fowl during the pullet year. Poultry Sci. 7: 245.

Duncan, D. B., 1955. Multiple range and multiple F tests. Biometrics 11: 1.

Dunnicliff, A. A., Jr., 1913. Ten years egg laying tests at Hawkesbury Agricultural College and Experimental Farm. Richmond, New South Wales. New South Wales Dept. Agr. Farmers Bul. 66: 1-96.

Edgar, S. A., 1954. Effect of temperature on the sporulation of oocysts of the protozoan, Eimeria tenella. Trans. Amer. Micros. Soc. 73: 237.

Edgar, S. A., 1955. Sporulation of oocysts at specific temperatures and notes on the prepatent period of several species of avian coccidia. Jour. Parasit. 41: 214-216.

Ehleres, V. M. and W F. Steel, 1927. Municipal and rural sanitation. McGraw-Hill Book Co. p. 87.

Emmel, M. W., 1930. Bacterial flora in feces of the normal fowl. J. Infectious Diseases 46: 293.

Enigh, C. F., 1935. Arch. Wiss. u. Prakt Tierheilk 69: 410.

Fabricant, J., P. P. Levine, B. W. Calnek, H. E. Alder and J. R. Berg, 1959. Studies of egg transmission of PPLO in chickens. Avian Diseases 3: 197.

Faddoul, G. P. and R. C. Ringrose, 1950. Avian keratoconjunctivitis. Vet. Med. 45: 492.

Fair, M. G., J. C. Geyer and J. C. Morris, 1954. Water supply and waste disposal. John Wiley and Sons, Inc., New York.

Forgacs, J. and W. T. Carll, 1955. Preliminary mycotoxic studies on hemorrhagic diseases in poultry. Vet. Med. 50: 172.

Forgacs, J., H. Koch and W T. Carll, 1955. Further mycotoxic studies on poultry hemorrhagic disease. Poultry Sci. 34: 1194.

Forgacs, J., W. T. Carll, A. S. Herring and B. G. Mahlandt, 1954. A toxic Aspergillus clavatus isolated from feed pellets. Am. J. Hyg. 50: 15-26.

Forgacs, J., H. Koch, W. T. Carll and R. H. White-Stevens, 1958. Additional studies on relationship of mycotoxicoses to the poultry hemorrhagic syndrome. Am. J. Vet. Res. 19: 744-753.

Forgacs, J., H. Koch, W. T. Carll and R. H. White-Stevens, 1962. Mycotoxicoses. I. Relationship of toxi fungi to moldy feed toxicosis in poultry. Avian Dis. (accepted for publ. Feb. 7, 1962).

Gage, G. E., 1911. A study showing bacterial and animal organisms determined in the feces and intestinal mucosa of healthy chickens. Md. Agr. Exp. Sta. Bul. 153, p. 201.

Gardiner, J. L., 1955. The severity of cecal coccidiosis infection in chickens as related to the age of the host and the number of oocysts ingested. Poultry Sci. 34: 415.

Gross, L., 1951. The "Vertical" transmission of mouse mammary carcinoma and chicken leukemia. Its possible implications for human pathology. Cancer 4: 626.

Gross, L., 1952. Mouse leukemia. Viruses as causative agents in cancer. Ann. N. Y. Acad. Sci. 54: 1184.

Gross, W. B., 1961. The development of "Air sac disease". Avian Dis. 5: 431.

Halbrook, E. R., 1950a. Vitamin B_{12} synthesis by microorganisms in poultry house litter. Ph.D. Thesis, Ohio State Univ.

Halbrook, E. R., 1950b. Proc. 39th Ann. Meeting of Poultry Sci. Ass'n. p. 16.

Halbrook, E. R., T. S. Sutton and A. R. Winter, 1950. The effect of management on the vitamin B_{12} content of poultry house litter. II. As determined by microbiological assay. Poultry Sci. 29: 679.

Halbrook, E. R., T. S. Sutton and A. R. Winter, 1951. The microflora of poultry house litter and droppings. Poultry Sci. 30: 381.

Hammond, J. C., 1942. Cow manure as a source of certain vitamins for growing chickens. Poultry Sci. 21: 544.

Harris, J. A. and C. D. Boughton, 1927. The death rates of three standard breeds of fowl. Poultry Sci. 7: 120.

Hays, F. A., 1955. Age of parents and mortality in off-spring. Poultry Sci. 34: 473.

Hays, F. A. and E. W. Spear, 1952. Relation of age of parents to maturity and sex ratio of chicks to eight weeks. Poultry Sci. 31: 792.

Helmboldt, C. F., F. K. Wills and M. N. Frazier, 1963. Field observations of the pathology of skin leukosis in Gallus Gallus. Avian Dis. 7: 402.

Herrick, C. A., 1934. The development of resistance to the protozoan parasite, Eimeria tenella by the chicken. J. Parasit. 20: 329.

Hinshaw, W. R., 1952. How diseases become established in poultry flocks. Vet. Med. 47: 164.

Hutt, F. B., 1951. The control of leukosis in the fowl. World's Poultry Sci. J. 7: 16.

Hutt, F. B., and R. K. Cole, 1954. Problems concerning leukosis and its control. Proc. 10th World's Poultry Congr., Edinburgh, Scotland 197.

Hutt, F. B., R. K. Cole, M. Ball, J. H. Bruckner and R. F. Ball, 1944. A relation between environment to two weeks of age and mortality from lymphomatosis in adult fowls. Poultry Sci. 23: 396.

Jaquette, D. S. and D. E. Fogg, 1962. Survey of poultry diseases on Del Marva Peninsula. Broiler Producer, Mar., 1962, p. 18.

Jull, M. A., 1934. The mortality problem in poultry. A reprint from the July and August, 1934 issue of the U. S. Egg and Poultry Mag. pp. 11-

Kennard, D. C., 1933. Pullet mortality. Poultry Sci. 12: 335.

Kennard, D. C. and V. D. Chamberlin, 1934. Poultry mortality. Ohio Agr. Expt. Sta. Bi-monthly Bul. 19: 137.

Kennard, D. C. and V. D. Chamberlin, 1936. Eight years experiences with losses of pullet layers. Ohio Agr. Expt. Sta. Bi-monthly Bul. 21: 63.

Kennard, D. C., R. M. Bethke and V. D. Chamberlin, 1948. Built-up floor litter a source of dietary factors essential for hatchability of chicken eggs. Poultry Sci. 27: 477.

Kennard, D. C. and V. D. Chamberlin, 1947. Lime treatment of floor litter for chickens. Ohio Agr. Expt. Sta. Farm and Home Res. 32: 11.

Kennard, D. C. and V. D. Chamberlin, 1948a. Built-up litter as a source of dietary factors essential for the growth of chickens. Poultry Sci. 27: 240.

Kennard, D. C. and V. D. Chamberlin, 1948b. Built-up floor litter to date. Ohio Agr. Expt. Sta. Farm and Home Res. 33: 130.

Kennard, D. C., V. D. Chamberlin, 1949. Built-up floor litter sanitation and nutrition. Ohio Agr. Expt. Sta. Farm and Home Res. 34: 162.

Kennard, D. C., V. D. Chamberlin, 1950a. Proc. 39th Ann. Meeting Poultry Sci. Ass'n.

Kennard, D. C. and V. D. Chamberlin, 1950b. Mortality of chicks as affected by floor litter. World's Poultry Sci. Jour. 6: 183.

Kennard, D. C., 1950c. Floor litter management as a factor in poultry nutrition. World's Poultry Sci. Jour. 6: 177.

Kennard, D. C. and V. D. Chamberlin, 1951. Growth and mortality of chickens as affected by the floor litter. Poultry Sci. 30: 47.

Kern, H., 1897. Beitrag zur kenntris der im drama und magender vagel. Vorkommender Bactrien Arbeiten Bact. Ins. Tech. Hochschule Karlsruke 1: 379.

King, W. E., 1905. The bacterial flora of the intestinal mucosa and conjunctiva of the normal chicken. J.A.V.M.A. 10: 400.

Koutz, F. R., 1948. Immunity studies in avian coccidiosis. Poultry Sci. 27: 793.

Koutz, F. R., 1952. The effect of built-up litter on the parasitic ova and oocysts of poultry. I. Poultry Sci. 31: 123.

Koutz, F. R., 1953. The effect of built-up litter on the parasitic ova and oocysts of poultry. II. Poultry Sci. 32: 320.

Lamoreux, W. F. and W Schumacher, 1940. Is riboflavin synthesized in the feces of fowls. Poultry Sci. 19: 418.

Lippincott, W. A. and L. E. Card, 1946. Poultry Production. Lea and Febiger, Philadelphia, p. 292.

Mackie, T. J. and J. E. McCartney, 1950. Handbook of Practical Bacteriology. Eighth ed. E. and S. Livingstone, Ltd. Edinburgh. p. 233.

Marr, J. E., C. W Pope, H. L. Wilcke and R. M. Bethke, 1961. Re-evaluation of the phosphorus requirement of the laying hen. Poultry Sci. 40: 1427.

McGinnis, J. J., M. Stevens and K. Groves, 1947. The in vitro synthesis of a chick growth promoting factor in hen's feces. Poultry Sci. 26: 432.

Misner, E. G., 1932. Economic studies of poultry farming in New York. IV. Cornell Agr. Expt. Sta. Mimeo Reports.

Moore, E. N. and V. D. Chamberlin, 1953. Compost litter. Nulaid News 31: 18 (Sept.)

Morgan, B. B. and P. A. Hawkins, 1952. Veterinary Protozoology. Burgess Publishing Co., Minneapolis, Minn. 187 pp. 23 plates.

Morris, D., 1963. Condemnations in poultry. The Merck Agricultural Memo. 8(4): Nov.

Neal, W. M., 1956. Cannibalism, pickouts and methionine. Poultry Sci. 35:10.

New York Random Sample Poultry Tests, 1953. (Three-year average report) College of Agriculture, Cornell Univ.

Olesiuk, O. M. and H. Van Rockel, 1960. Transmission of chronic respiratory disease in chickens. Avian Disease 4: 348.

Patterson, F. D., H. L. Wilcke, C. Murray and E. W. Henderson, 1932. So-called range paralysis of the chicken. Am. Vet. Med. Ass'n. J. 34: 747.

Profitable Management, 1963. A series of Beacon research, May 14. Beacon Feeds, Beacon Division of Textron, Cuyaga, N. Y.

Raggi, L. G., J. R. Brownell and G. F. Stewart, 1961. Effects of infectious laryngotracheitis virus on egg production and quality. Poultry Sci. 40:134.

Random sample egg production test report. United States and Canada (1963). Agr. Res. Service, U. S. Dept. of Agr.

Reid, W. M. T. A. Maag, M. B. Frank, A. L. Kleckner and S. C. Schmittle, 1961. Embryo and baby chick mortality and morbidity induced by a strain of E. coli. Poultry Sci. 40: 1501.

Rubin, M., H. R. Bird and I. Rothchild, 1946. A growth promoting factor for chicks in feces of hens. Poultry Sci. 26: 439.

Schumacher, A. E., G. F. Heuser, 1941. Bacterial flora in chicken feces. Poultry Sci. 20: 272.

Scudder, H. D., A. S. Burrier, A. G. Lunn and F. L. Knowlton, 1931. Cost and efficiency in commercial egg production in Oregon. Ore. Agr. Expt. Sta. Bul. 287: 1.

Sevoian, M. and D. M. Chamberlin, 1963. Avian lymphomatosis. III. Incidence and manifestations in experimentally infected chickens of various ages. Avian Diseases 7: 97.

Sevoian, M., D. M. Chamberlin and R. N. Larose, 1963. Avian lymphomatosis. V. Air-borne transmission. Avian Diseases 7: 102.

Sheppard, C., 1964. Personal communication. Extension Service Michigan State University.

Singsen, E. P., A. H. Spandorf, L. D. Matterson, J. A. Serafin, 1961. Phosphorus in the nutrition of the adult hen. Minimum phosphorus requirements. Poultry Sci. 40: 1457.

Smith, R. C., 1956. Kind of litter and breast blisters on broilers. Poultry Sci. 35: 595.

Snedecor, G. W., 1956. Statistical Methods Applied to Experiments in Agriculture and Biology. Fifth ed., Ames Iowa College Press.

Snoeyenbos, M., M. Sevoian, D. L. Anderson and H. I. Basch, 1961. Avian Diseases 1: 349.

Snyder, J. M., O. A. Rowath, J. C. Schales, C. E. Lee, 1962. Profitable Poultry Management. 24th ed. Beacon Feeds.

Squibb, R. L., 1961. Avian diseases virus and nutrition relationships. Poultry Sci. : 425.

Stafseth, H. J., W. W. Thompson and C. G. Grey, 1932. Observations on prolapse (Blow-outs). Jour. Am. Vet. Med. Ass'n. 80: 80.

Stafseth, H. F. and E. S. Weisner, 1931. Causes of mortality in laying hens. Mich. Agr. Expt. Sta. Quart. Bul. 13: 153.

Sunde, M. W., W. W. Cravens and J. G. Halpin, 1951. The effect of vitamin B_{12}, antibiotics and deep litter on laying and breeding hens. Poultry Sci. 30: 932.

Takashi, O. and W. M. Reid, 1961. Histomoniasis in chickens. Age of greatest susceptibility and pathogenecity studies. 5: 355.

Taylor, L. W., D. C. Lowery and L. G. Raggi, 1955. Effect of an outbreak of avian encephalomyelitis (Epidemic tremor) in a breeding flock. Poultry Sci. 34: 1037.

Thomas, W. P. and M. Clawson, M., 1933. Economic factors affecting poultry production and marketing in Utah. Utah Agr. Expt. Sta. Bul. 244: 1.

Titus, W., D. W. Whitson and H. R. Bird, 1945. The effect of feeding cow manure on egg production and hatchability. Poultry Sci. 25: 52.

Todd, A. C., 1948. Worm parasites of Tennessee chickens. Tenn. Agr. Exp. Sta. Bul. 205.

Tyzzer, E. E., E. H. Theiler, and E. J. Jones, 1932. Coccidiosis in gallinaceous birds. II. A comparative study of species of Eimeria of the chicken. Am. J. Hyg. 15: 319.

Van Rockel, H., 1955. Advances in Veterinary Science 2: 64.

Voorhies, E. C. and G. A. Read, 1931. A biometrical study of the mortality of Single Comb White Leghorn chicks. Hilgardia 5: 531.

Waters, N. F., 1951. Mortality from lymphomatosis and other causes among inbred lines of White Leghorns. Poultry Sci. 30: 531.

Waters, N. F. and C. O. Prickett, 1944. The development of families of chickens free of lymphomatosis. Poultry Sci. 23: 321.

Waters, N. F. and J. H. Bywaters, 1949. Influence of age of chickens at contact exposure on incidence of lymphomatosis. Poultry Sci. 23: 321.

Waters, N. F. and J. H. Bywaters, 1949. Influence of age of chickens at contact exposure on incidence of lymphomatosis. Poultry Sci. 28: 254.

Wheeler, R. S. and E. Hoffmann, 1948. Prolapse and pickouts in diethyl-stilbestrol-treated cockerels. The Cornell Vet. 38: 89.

Whitson, D. W., W. W. Titus and H. R. Bird, 1945. The effect of feeding cow manure on egg production and hatchability. Poultry Sci. 25: 52.

Winton, B., 1932. Poultry sanitation program for Missouri. Missouri Agr. Expt. Sta. Ext. Cir. Bul. 282.

1959
Wright, M. L., G. W. Anderson and N. A. Epps./ Hatchery sanitation. Canad. Jour. Comp. Med. 23: 288.

Wright, M. L., G. W. Anderson and N. A. Epps, 1960. Hatchery sanitation as a control measure for Aspergillosis in fowl. Avian Diseases 4: 368.

Wright, M. L., G. W. Anderson and J. D. McConachie, 1961. Transmission of Aspergillosis during incubation. Poultry Sci. 40: 727.

Yates, J. D., 1963. The feeding of unheated soybeans to poultry. Ph.D. Thesis. Michigan State University.

Young, B. P. 1929. A quantitative study of poultry coccidiosis. J. Parasit. 15: 241-250.

Appendix Table 1. Composition of Standard Chick Starter* (M.S.U. 63-S) Used in Experiments I and II (Amts. in lbs/2,000 lbs.)

Ingredient	Pounds
Soybean oil meal, 45% protein	500
Corn, ground yellow	1060
Oats, ground	100
Wheat flour middlings	100
Alfalfa meal, dehyd., 17% protein	80
Meat and bone meal, 50% protein	50
Whey, dried	40
Fish meal, 55% Vitaproil	40
Limestone, ground	10
Dicalcium phosphate (24% Ca., 18.5% Phos.)	10
Salt, iodized	6
Vitamin trace mineral	5
Coccidiostat (Zoamix)	1

Manufactured by King Milling Company, Lowell, Michigan

Appendix Table 2. Residual Chlorine Determinations in Filtrate From Mixture of Feed and Chlorinated Water

Amount of feed (gms)	Amount of water (mls)	Chlorine levels in water (ppm)	Residual chlorine after time in mins. 5	10	15	20	30	45
5	10	500	1	0	-	-	-	-
30	60	"	-	-	-	-	-	-
30	70	"	5	-	-	-	-	-
32	70	"	1	0	-	-	-	-
30	70	1000	10	0	-	-	-	-
30	70	"	5	0	-	-	-	-
30	80	"	50	5	0	-	-	-
32	100	"	100	10	6	1	0	-
32	120	"	250	80	5	1	0	-

0 - No available chlorine

Appendix Table 3. Composition of Ration (Amounts in lbs/200 lbs.) Used in Experiments III, IV and V

Ingredients	Pounds
Ground corn	1203
Pulverized oats	100
Soybean oil meal, 44%	300
Corn gluten meal	100
Fish meal, 60%	50
Meat and bone scraps	50
Alfalfa meal, dehyd., 17%	60
Dried whey	35
Dried brewers yeast	35
Ground limestone	30
Dicalcium phosphate	25
Salt (iodized)	5
Choline chloride, 25% dry mix	2.5
Vita. A, D & E premix[1]	2
Coccidiostat[2]	1
Arsanilic acid[3]	1
Manganese sulfate, feed. grade	0.5
Antioxidant[4]	0.25
Niacin	18 grams
Procaine penicillin	2 grams

1
2 Hamilton Farm Bureau
3 Hess and Clark, potency 50 gms/lb.
4 Abbott Laboratories, Pro-gen 90 gms/lb.
BHT

Appendix Table 4. Composition of Michigan State University Z-4 Battery All-mash* Used in Experiments VI, VII and VIII (Amounts in lbs./2000 lbs.)

Ingredient	Pounds
Soybean oil meal, 44% protein	50
Corn, ground yellow	690
Oats, ground	400
Bran	300
Alfalfa meal, dehydrated, 17%	60
Wheat flour middlings	200
Milk, dried	40
Fish meal	50
Meat scraps	60
Oyster shell flour	100
Bone meal (steamed)	30
Salt, iodized	12
Fish oil	8

* Manufactured by King Milling Co., Lowell, Michigan.

Appendix Table 5. Composition of Ration for Baby Chicks Used in Experiment X

Ingredients	Percent
Soybean oil meal, 44% protein	31.4
Corn, ground yellow	50.8
Fish meal and solubles, 57% protein	3.0
Alfalfa leaf meal, 20% protein	2.0
Corn oil	6.6
Dried distillers solubles, corn	2.0
Vitamin-trace mineral mix, Nopcosol M-7	0.25
Whey, dried	2.0
Salt, iodized	0.50
Limestone, ground	0.90
Dicalcium phosphate	0.60

Calculated analysis	%
Protein	21.92
Fat	9.22
Fiber	3.64
Calcium	0.82
Phosphorus	0.61
Arginine	1.26
Glycine	1.17
Methionine	0.43
Cystine	0.33
Lysine	1.18
Tryptophan	0.25
Productive energy Cal/lb	1024

Appendix Table 6. Composition of Vitamin-trace Mineral Mixes* (per 5 lb.)

	Nopcosol M-4	M-7
Vitamin A, USP units	4,000,000	5,000,000
Vitamin D_3, IC units	1,500,000	1,500,000
Vitamin E, I units	7,500	5,000
Riboflavin	3	3
d-Pantothenic acid, gm	5	5
Niacin, gm	20	30
Choline chloride, gm	200	300
Menadione sodium bisulfite, gm	--	--
Vitamin B_{12}, gm	10	6
Zinc bacitracin, gm	--	4
Butylated hydroxy toluene, gm	113.4	113.4
Manganese, %	2.4	2.4
Zinc, %	0.8	2.2
Iron, %	0.8	0.8
Copper, %	0.08	0.08
Iodine, %	0.048	0.048
Cobalt, %	0.008	0.008

* Produced by Nopco Chemical Company, Harrison, New Jersey

Appendix Table 7. Post Mortem Diagnosis Sheet*

I. Hen Number_____, Age_____, Pen Number_____, No. birds in pen_____, No. birds affected in each pen _____, No. bird dead_____, Symptoms observed, if any _____

_____

Time of death (before post mortem)_____

Physical Examination_____

II. Droopinessn, Lameness, Blindness, Emaciation

Discoloration of comb and wattles_____

Condition of nasal sinuses and mouth_____

External parasite, if any_____

Body temperature_____

III. (Visceral Examination)

1. Intestines: Color_____, Thickness of wall_____, Tuberculi noduli, if any _____, Hemorrhages_____, Worms_____

Gizzard and Crop: Odor of contents. Congestion of, if any lesions present_____.

Esophagus and Trachea: Color_____, Noduli, if any_____

Heart: Any abnormality_____

Liver: Color_____, Any noduli_____,

Fatty degeneration_____,Necrosis_____,

Kidneys: Color_____, Enlargement or swelling, if any_____

Presence of calculi_____.

Lungs: Color_____, Noduli, if any_____

Testes:_____

Ovaries:_____

Oviduct:_____

IV A. Macroscopic Findings_____

B. Microscopic Findings:_____

C. Conclusions:_____

* All birds were autopsied according to this postmortem sheet.