INFLUENCE OF EGG SIZE, EGGSHELL QUALITY, AND HATCH AND

PLACEMENT TIMES ON THE PERFORMANCE OF BROILER CHICKENS

by

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INTRODUCTION

In the poultry industry today, economic pressures require producers to raise broilers in an efficient manner. The quality of chicks provided to these producers can materially influence performance at market age. To best utilize labor resources and to ensure maximum hatch, hatcherymen remove chicks only at the end of the hatching cycle (normally 21 days, 12 hrs). This practice allows some chicks to remain in the incubator at a temperature > 37° C for a long period of time. It has been reported that exposure of chicks to high environmental temperatures prior to an antigenic stimulus from a vaccine may result in an inhibition of the primary immune response (Cheville, 1978). Consequently, studies are needed to determine the significance of hatcher induced stress and to see if losses in overall performance justify a change in hatchery management procedures.

Post-Hatch Holding Time On Performance

Time required for complete embryonic development can vary considerably in a typical commercial incubator. Genetic differences, preincubation storage time, season, egg size, eggshell quality, incubation temperature and humidity can influence this variation and cause the hatch time of individual eggs to occur over many hours.

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The difference between initial egg weight and placement weight of the newly hatched chick provides a measurement of dehydration during the incubation process. Rahn and Ar (1974) indicated that the incubation time for an egg is inversely proportional to the water vapor conductance of the egg shell. The energy needs of the growing embryo are supplied by the fat stores in the yolk. For every gram of fat used, approximately one gram of metabolic water is produced. Consequently, the relative water content of an egg will increase unless water is passed out of the shell. Rahn et al. (1979) reported that approximately 15% of the initial mass of the egg must be lost as water in order to insure a successful hatch and prohibit drowning of the chick within the shell.

As time following hatching and preceding removal from the hatcher increases, the percentage of water loss increases in the form of dehydration. Thaxton and Parkhurst (1976) reported the importance of hydration in newly hatched broiler chicks during brooding on early mortality and growth. They found that chicks fed water or water that contained 10% sucrose 12 hrs prior to being placed on feed had significantly heavier body weights through 8 wks of age than hatchmates that received water and feed simultaneously. Houpt (1958) demonstrated that body weight loss is proportional to the length of time the newly hatched chick

is deprived of feed.

Due to the standard commercial practice of removing all
chicks from the hatcher at one time, many of the chicks
remain in the hatcher for long periods of time before
removal. Henderson and Champion (1948) found that chicks
hatched and removed early from the hatcher had a tendency to
be heavier at eight weeks of age than late hatching chicks.
Caylor and Laurent (1961) reported that chicks placed
between 22 and 32 hrs after hatching had lower body weights
and higher feed conversions at eight weeks of age than
hatchmates placed in brooding pens 12 hrs after hatch. Hill
and Green (1977) found that chicks held in the incubator for
48 hrs instead of being placed directly into growing pens
with feed and water had significantly lower body weights at
28 days of age. A study conducted by Moran and Reinhart
(1980) found that poults were significantly heavier through
2 wks of age when removal from the hatcher was performed
twice as compared to one complete removal at the end of the
hatch period. Males in the study maintained this
relationship through 10 wks of age. In addition to delayed
removal time from the hatcher causing growth depression,
Andrews (1974) and Carson (1975) reported that this practice
increased mortality among these chicks. Twining et al.
(1978) reported that birds placed in floor pens and provided
water 4 hrs after removal from hatchers were significantly

heavier at 28, 49, and 56 days of age than birds removed from hatchers and held in the hatchery 24 hrs prior to placement.

Studies by Hess and Dembnicki (1962) and Conner et al. (1971) reported that holding chicks in boxes for 36 to 72 hrs after hatch had no significant effect on 8 week or 10 week body weights, respectively, when compared with chicks placed in pens soon after removal from the hatcher. However, Hess and Dembnicki (1962) found that mortality was increased in chicks deprived of feed and water for 72 hrs after hatch.

Egg Size and Eggshell Quality On Performance

In addition to the length of time chicks are held in the incubator post-hatch, egg size and eggshell quality can also affect the amount of weight loss incurred by chicks during incubation. Godfrey and Williams (1952) found that 74% of the variation in body weight at market age for broilers is due to egg size, age of parents at sexual maturity and mature body size of the parents. They reported that egg size caused the greatest influence. Williams et al. (1951) and Goodwin (1961) reported that chick weight at hatch was positively correlated with egg weight, with this relationship reflected on subsequent growth rates to market age. McNaughton et al. (1978) found heavier broiler market

weights in chicks hatched from either 57-62 or 67-74g eggs
when compared with chicks hatched from 47-54g eggs. An
experiment conducted by Whiting and Pesti (1984) reported
that each additional gram of egg weight at time of
incubation corresponded to 10.7 and 6.0 g in additional
final body weight for broiler males and females,
respectively. They explained the influence of egg size on
broiler body weight as an indication of the genetic profile
of the dam. Females laying larger eggs may have a superior
genetic profile for increased size and growth. Furthermore,
Halbersleben and Mussehl (1922) and Gardiner (1973) reported
that the influence of egg size on chick body weight was
evident early in life but declined with age. In the study
by Gardiner (1973), females from larger (>56g) versus
smaller (<54g) eggs had heavier body weights through 8 wks ;
with males from the larger egg size group heavier through 6
wks of age. Studies by Upp (1928), Godfrey et al. (1953)
and Bray and Iton (1967) concluded that the influence of egg
size on chick weight was not highly correlated after
approximately 2 wks of age.

Another factor which may be influenced by egg size is
chick mortality. McNaughton et al. (1978) found that a 7%
higher mortality occurred when chicks were hatched from eggs
laid by 29 week old breeder hens when compared with eggs
from 58 week old breeder hens. Also, these researchers

found chicks that hatched from small eggs (<54g) had a
higher mortality rate than chicks from large eggs (>58g).
Hays (1955) reported that chicks from older parents were
more viable than progeny from younger parents. O'Neil
(1955) showed that chick weights which were a smaller
percentage of the original egg weight at hatch had a high
mortality rate early in life. However, other studies have
found no relationship between egg weight and mortality
(Skoglund and Tomhave, 1949; Wiley, 1950; Tindell and
Morris, 1964).

Chick weights at hatch may be influenced by age of the
broiler breeder hen. Reinhart and Hurnik (1984) found that
chick weight at hatch was influenced by breeder age and egg
weight. However, a study by McNaughton et al. (1978) found
no differences in chick weights due to the age of parents
when egg weights were similar. Consequently, these
researchers concluded that hatching egg size and not age of
parents limited chick weights at hatching.

Previous studies have shown that eggs weighing less than
52g hatch earlier than eggs weighing more than 65g
(Henderson and Champion, 1948; Williams et al., 1951).
Reinhart and Hurnik (1984) found that hatching time was
influenced by egg weights and age of breeder flocks, with
the younger flock producing smaller eggs which exhibited the

shorter incubation period.

It has been hypothesized that poor eggshell quality may cause lower hatchability and early chick mortality. Many factors can affect shell texture and strength, such as age of hens, genetic background, plane of nutrition and environmental conditions. Specific gravity estimates eggshell mass and is considered a reliable indicator of shell porosity and breaking strength (Potts et al., 1974; and Hamilton, 1982).

Studies by Munro, (1940), Coleman and McDaniel, (1975) and McDaniel and Brake (1981) reported that eggs with a specific gravity < 1.080 had lower hatchability and higher early and late embryonic mortality than eggs with a specific gravity > 1.080. These researchers found that eggs with more porous shells displayed greater weight loss through water evaporation during incubation than eggs with less porous shells. Mussehl and Halbersleben (1923) found a slight positive correlation between specific gravity and hatchability, but no relationship between specific gravity and chick viability at hatch or growth rate during the first 5 wks of age. However, Mueller and Scott (1940) found that egg weight loss which, is correlated with egg specific gravity, had no influence on hatchability.

Hatching eggs with poor shell quality are thought to increase water evaporation and dehydration among chicks during the incubation. Tullet and Burton (1982) found that over 97% of the variation in chick weight when compared with egg weight at hatch can be explained through the amount of egg weight loss during incubation. Ar and Rahn (1980) demonstrated that weight loss during incubation comes entirely from water vapor loss and not from the loss of other metabolites. O'Neil (1955) reported that broiler chick weights were heavier and had lower mortality at 6 wks of age when these chicks represented a larger percentage of the initial egg weight at hatch.

Immune Response To Stress

A major question among poultrymen is how much influence does the environment in the early life of the chick have on its initial response to an antegin and consequently, on its general health and productivity. Environmental factors such as feed and water deprivation, and excessive heat or cold which may occur at placement time, can influence the immune response of birds (Cheville, 1978). He reported this influence to occur because of the ability of adrenal glands to synthesize and secrete corticosteroids which suppress antibody forming cells. This pathway includes water deprivation and heat or cold stress. Morgan (1980)

classified the chicken as steroid sensitive, since glucocorticoids cause physiological changes such as reductions in bursa and spleen weights (Glick, 1967) and suppression of humoral immune responses (Sato and Glick, 1970). Therefore, environmental stresses may result in several changes that are termed "adaptation reactions" (Siegel, 1971). These include 1) lymphatic involution; 2) white blood cell alterations; 3) changes in blood chemistry such as ions, cholesterol, nitrogenous products or sugar levels; 4) gastro-intestinal ulceration; 5) anti-inflammatory action and 6) antibody activity. Hill (1983) reported that physiological changes in the bird may be a good indicator of stress. Following is a discussion of several of these changes.

Lymphatic tissue- The bursa of Fabricius and spleen are two lymphatic tissue areas in the chicken. The bursa of Fabricius is an endocrine gland of the bird responsible for the control of circulating antibody mediated immunity. This gland is responsible for regulating the level of circulating immunoglobulins, and production of antibodies (Glick, 1978). The normal growth of the bursa is more rapid than that of the total body during the first three weeks of age. The growth of this organ slows after this period and begins to regress between five and eight weeks of age. Several researchers have shown that the growth of the bursa is

reduced and may actually atrophy when the bird is exposed to stressful conditions (Glick, 1956; Garren and Shaffner, 1956; Huble, 1958; Siegel, 1961). Garren and Shaffner (1956) found that a decrease in the weight of the bursa can be used as an index for measuring lymphatic involution during stress. It is postulated that when a bird is exposed to environmental stressors, the adrenal cortex releases corticosterone which acts directly on lymphatic tissues. These tissues are found in the thymus, spleen, and bursa of Fabricius. Researchers have found that injections of ACTH or exposure to low temperatures cause the bursa to decrease in size (Garren and Shaffner, 1956; Newcomer and Connally, 1960; Siegel and Beane, 1961; Siegel, 1961).

The spleen is a lymphoid organ associated with the circulatory system of the bird. Glick (1967) reported that the normal development of the spleen is related to bursa development. In the same study, he found injections of cortisone acetate (7.5 mg) in young chickens depressed normal spleen development which resulted in a depression of antibody production. A study by Siegel (1961) demonstrated that ACTH injections significantly depressed the growth of the spleen. Siegel and Gould (1979) found that a one hour exposure to high temperatures resulted in an increased amount of corticosteroid taken into the nuclei of lymphoid tissue cells. The exposure of birds to acute high

temperatures along with an injection of ACTH has been demonstrated to exert a strong immunosuppressive effect (Thaxton et al. 1968). The exposure of chicks to high temperatures may result in circulating levels of steroids acting directly on the lymphoid tissues, such as the bursa and spleen. Therefore, disease resistance in general may be associated with bursal growth and size during the early critical period when chicks first develop the capacity to produce antibodies (Glick et al., 1956).

Blood chemistry- Different environmental stressors can influence circulating levels of blood glucose and proteins. Stress can affect the metabolism of birds by stimulating the release of neurogenic amines (epinephrine) which influence the ability of the liver to breakdown glycogen to glucose. Also, corticosteroids can activate liver glycogenolysis and increase the blood glucose level (Snedecor et al.,1963). Brown et al.(1958) demonstrated that the increase in glucose levels during stress is a result of the breakdown of protein. Therefore, this protein breakdown may result in a decrease of total proteins and an increase in non-protein nitrogen in the blood. This shift in metabolism provides the bird with resources to deal with environmental stress.

Siegel (1971) reported an increase in glucose levels and a decrease in total proteins during heat exposure. A study

by Edens and Siegel (1976) showed plasma glucose and
corticosterone levels to be increased in two week old chicks
during acute heat exposure.   Jones et al.(1981) indicated
plasma protein significantly decreased, while plasma glucose
significantly increased following injections of endotoxins.
Therefore, they postulated that these changes occur due to
an increase in catabolism of protein and fatty tissue
through gluconeogenesis.   This increased catabolism of
antibodies for energy is increased by the involvement of
glucocortcoid hormones.

A major factor affecting the chick during delayed
placement is dehydration.   A physiological parameter used to
indicate blood viscosity or dehydration is the hematocrit
(packed cell volume).   Christensen et al.(1982) observed a
significant increase in pack cell volume at hatching time
and attributed it to dehydration during the change from
chorioallantoic to pulmonary ventilation and its associated
evaporative effect.   Chamblee and Morgan (1983) found that
hematocrits increased within 24 hrs after the removal of
feed and/or water.

Blood cells- In addition to the metabolic changes that
occur with stress, corticosteroids have been shown to
influence the number of lymphocytic and heterophilic white
blood cells present in the blood.   Chancellor and Glick

(1960) found that birds exposed to high temperatures had a
marked increase in the percentage of heterophils and a
decrease in the percentage of lymphocytes in the blood.
Furthermore, researchers have found that acute physiological
stressors, such as ACTH or cortisone acetate injections,
alter the differential leucocytic haematology of the bird(
Glick, 1958; Newcomer, 1957 and 1958; Wolford and Ringer,
1962; Bhattacharyya and Sarkar, 1968; Siegel, 1968). Gross
and Siegel (1983) found the number of lymphocytes to
decrease and the number of heterophils to increase in
chicken blood in response to fasting, Escherichia coli or
Newcastle disease vaccine challenges and to increased
corticosterone levels in the feed. They found the
heterophil/lymphocyte ratio to be less variable than the
number of heterophil or lymphocyte cells in the blood, and
reported that an elevation of these cells was a good measure
of bird response to environmental stress.

It is thought that the binding of circulating
corticosteroids to the lymphoid cells causes cell
destruction and reduces the production of lymphocytes.
Glick and Sato (1964) found that the absolute count of
lymphocytes was significantly lower in bursectomized birds
when compared with controls. They postulated that the bursa
of Fabricius is necessary for the production of optimal
levels of circulating lymphocytes in the bird. A study by

Vo and Fanguy (1982) found temperature stressed birds to
have a suppression in humoral immunity with a corresponding
reduction in primary and secondary humoral immunity titers.
Therefore, environmental heat stressors that increase the
level of circulating adrenal steroids appear to act directly
on lymphoid tissues to reduce the immune responsiveness of
the bird (Thaxton, 1978).

GENERAL MATERIALS AND METHODS

Two experiments (Exp 1 and Exp 2) were conducted
utilizing hatching eggs collected from two commercial
broiler breeder stocks. The eggs were weighed and placed in
an egg room at 16° C and 70% relative humidity (R.H.).
Prior to storage, eggs in Exp 2 were measured for specific
gravity. The body weight and feed efficiency portion (part
1) and the immune response portion (part 2) of Exp 2 were
conducted at different times of the year and used different
specific gravity levels. After 7 days in storage, eggs were
reweighed to determine storage weight loss and placed in
Petersime incubators at 37.6° C and 55% R.H. On the 18th
day of incubation, the eggs were candled and the fertile
eggs were transferred to Petersime hatchers at 37.5° C and
65% R.H. Eggs in Exp 2 were segregated in hatching trays by
egg weights and specific gravities. A plastic room (Figure
1) was positioned in front of the hatcher doors on the 19th
day of incubation. The environment inside this room was
maintained at 35° C and 55% R.H., and provided a work area
where chicks were wingbanded and weighed prior to being
placed back in the hatcher for the various holding periods.
Furthermore, hatcher doors were opened only long enough to
remove and replace trays.

The incubation period was divided into four hatch times

Figure 1. Plastic chamber used to enclose front of hatcher to control temperature and humidity

with approximately 25% of the chicks in each period. Hatch frequency was monitored starting on the 20th day of incubation, with the first hatch period containing chicks sufficiently dry enough for removal when approximately 18% of the total eggs set had hatched (estimating 70% hatchability). The succeeding hatch times were determined when the next group of chicks had hatched and were sufficiently dry for removal (Table 1).

Twenty-five chicks of each sex were placed in each of 28 floor pens that measured 1.52 X 3.66 m. This provided a stock density of .111 m² per bird. All pens were designed to control ambient temperature and eliminate outside light. Chicks were placed on litter which consisted of a combination of peanut hulls and pine shavings spread to a depth of 7 cm. All birds were provided 24 hrs of light at an intensity of 65 lux through 49 days of age. Temperature in all pens was maintained at 29.5° C for the first seven days of age. Then the temperature was reduced 3° C per week to 21° C at 28 days of age and maintained at this level for the remainder of both experiments.

Two commercial starter diets containing either 3135 or 3179 Kcal/Kg of metabolizable energy (ME) and 22.4 or 22.3% protein were provided ad libitum in crumble form from 1 to 28 days of age in Exp 1 and 2, respectively. Grower diets

Table 1. Removal times for each hatch period for Exps 1 and 2.
(hours of incubation)

	Hatch Period (hrs)*			
	1	2	3	4
Exp 1				
Large eggs (58-64 g)	484	492	499	516
Small eggs (48-54 g)	490	496	502	516
Exp 2				
High specific gravity ($\geq$1.080)	489	495	501	516
Low specific gravity ($\leq$1.070)	488	493	500	516

*Approximately 2 hrs were required to remove and weigh chicks in each hatch period.

in Exp 1 and 2 contained either 3190 or 3223 Kcal/Kg ME and 20.3 or 23.8% protein, respectively, and were fed ad libitum in pellet form from 29 to 42 days of age. Finisher rations had either 3190 or 3291 Kcal/Kg ME and 17.8 or 21.3% protein for Exp 1 and 2, respectively, and were fed ad libitum in pellet form for the final week of each experiment. Each pen was provided one tube feeder with a pan measuring 35 cm in diameter. This allowed 4.40 cm of feeder space per bird.

Chicks from the held and removed treatments in Hatch Period 1 were used to evaluate the influence of early and delayed removal from the hatcher on several physiological parameters.

Part 1

Influence of Egg Size, Eggshell Quality and
Post-Hatch Holding Times on Broiler Performance

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Introduction

A major factor that influences the success of a
commercial broiler hatchery is the quality of chicks
produced. The normal practice is to remove chicks from the
hatcher after approximately 21.5 days of incubation to
insure maximum hatch of fertile eggs. With this practice,
early hatching chicks remain in the hatcher for extended
periods of time prior to removal. Misra and Fanguy (1978)
reported that holding chicks in the hatcher for 32 to 48 hrs
post-hatch reduced chick placement weights. Since newly
hatched chicks possess limited food and water reserves, it
is necessary to provide these nutrients soon after hatching
to minimize physiological stress. A study by Houpt (1958)
found that body weight loss is positively correlated with
the length of time a chick is deprived of feed. Kingston
(1979) reported that chicks held in the hatcher for 48 hrs
after hatching were dehydrated and had significantly higher
10 day mortality than removed chicks.

Williams et al. (1951) found that chicks removed from the
incubator on the 20th day of incubation and placed on feed
and water grew slightly faster than late emerging chicks.
In the same study, chicks that hatched early but remained in
the hatcher were found to have a slower growth rate. Fanguy
et al. (1980) and Hager and Beane (1983) reported that

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chicks that had hatched by 504 hrs of incubation and left in the hatcher for an additional 18 hrs were significantly lighter at four weeks of age than removed chicks.

Hatching egg size and specific gravity have also been shown to affect broiler chicks and their market weights. Wiley (1950) and Tindell and Morris (1964) found a positive correlation between pre-incubation egg size and market broiler body weight. Furthermore, McDaniel et al. (1979) found that chicks from eggs with low specific gravity (< 1.080) had a greater weight loss, higher early mortality and lower hatchability than chicks from eggs with higher specific gravity (> 1.080).

This investigation was conducted to measure the influence of egg size, eggshell quality and post-hatch holding times in the hatcher on subsequent broiler performance.

Materials and Methods

Experiment I (Exp 1). Broiler hatching eggs were collected from a young breeder flock(26 wks of age) to obtain small eggs(47-54 g) and from an older flock(36 wks of of age) with a similar genetic background to obtain larger eggs(58-66 g). These eggs were weighed and placed in storage(16° C and 70% R.H.) for seven days then reweighed prior to incubation. The hatching sequence was divided into four periods with 25%

of the chicks removed in each period. All chicks
sufficiently dry in periods one through three were removed,
feather sexed, weighed and wingbanded. One-half of each
group was placed back in the incubator for additional
holding. The other one-half was vaccinated for Marek's
disease and held at 21° C for six hours prior to placement
in growing facilities. The remainder of the chicks held
from the first three groups were removed with group four at
21 days, 12 hours of incubation, weighed, vaccinated for
Marek's disease, and held for 6 hrs in chick boxes prior to
placement. This sequence provided seven separate hatching
groups.

Commercial husbandry practices were applied, and
commercially prepared starter, grower and finisher diets
were fed ad libitum during both experiments. Individual
body weights were recorded weekly and feed efficiencies
determined at 28 and 49 days of age. Percentage hatch of
all eggs set was recorded after incubation. However, only
1400 chicks were used in the growing phase with 25 males and
25 females placed in each of 28, 1.52 x 3.66 m pens.

Experiment II (Exp 2). This trial followed a format similar
to that used in Exp 1, with eggshell quality substituted for
egg size. Eggs were collected from two breeder flocks in
the latter stages of lay with similar genetic backgrounds.

Egg specific gravity was used as a measure of eggshell
quality (Potts et al., 1974). Eggs weighing 58 to 66g were
divided into high(≥1.080) and low(≤1.070) specific gravity
groups.

Statistical analyses. Mean differences within treatments
and interactions between treatments for each experiment were
determined by analysis of variance. Duncan's multiple range
test was used to seperate mean differences within treatments
when a significance level of P ≤ 0.05 was obtained with the
analysis of variance. Analyses were made using the
following statistical model:

$$Y_{ijkl} = u + T_i + E_j + S_k + (TE)_{ij} + (ES)_{jk} + (TS)_{ik} + (TES)_{ijk} + e_{ijkl}$$

where i = 1,2,3,4,5,6, and 7 hatch treatments (T), j = 1,2
egg size groups (Exp 1) or egg specific gravity groups (Exp
2) (E), k = 1,2 sex (S), and l = 1,2 . . . n birds, was used
to test differences in weekly body weights.
When significant, hatch treatments were separated using
orthogonal linear contrasts. Pen values for feed efficiency
were analyzed by analysis of variance with the model:

$$Y_{ijk} = u + T_i + E_j + R_k + (TE)_{ij} + e_{ijk}$$

where i = 1,2,3,4,5,6 and 7 hatching treatments (T), j = 1,2
egg size groups (Exp 1) or egg specific gravity groups (Exp
2) (E), and k = 1,2 replicates (R).

Mortality was analyzed with this same model after percentages were subjected to arc sin % transformation.

Results and Discussion

Egg size (Exp 1)

Egg size had a significant influence on male and female body weight through 49 days of age (Table 1). Chicks hatched from small eggs were 21.4% lighter at the time of placement than chicks from large eggs. A body weight difference between egg weight groups was maintained throughout the experiment; however, the influence of egg size progressively decreased during the growing period. Body weight of males hatched from 48 to 54 g eggs was 22% less at day 7, and 9% less by 49 days of age when compared to males from 58 to 64 g eggs. Females showed the same pattern with chicks from small eggs weighing 24% and 10% less at 7 and 49 days of age, respectively, when compared with females from large eggs. Even though the percentage difference decreased with age, the actual difference in weight was 188 and 173 g for males and females, respectively, at 49 days of age. Previous studies have reported similar results with chick weights being highly correlated to egg weight from placement to market age (O'Neil, 1955; McNaughton et al., 1978). Furthermore, Gardiner (1973) reported that egg weight influenced chick

Table 1. Infuence of egg size and egg specific gravity on broiler body weight and mortality

Egg Variable	Sex	Body Weights (g)						Mortality (49 days) (%)
		Start	7 days	21 days	28 days	35 days	49 days	
Size-								
Large (58-64g)	male	42.9^a	125.4^a	594.3^a	964.9^a	1400.9^a	2284.7^a	2.3^a
	female	42.6^a	124.5^a	541.6^b	843.3^b	1186.4^b	1875.1^b	2.0^a
Small (48-54g)	male	34.9^a	102.8^a	513.0^a	839.2^a	1240.1^a	2096.8^a	9.5^b
	female	34.9^a	99.7^b	466.4^b	735.3^b	1051.8^b	1701.9^b	6.7^b
Specific gravity-								
High (≥1.080)	male	43.4^a	131.2^a	629.0^a	1001.9^a	1484.5^a	2510.3^a	5.4^a
	female	43.2^a	127.5^b	568.9^b	882.9^b	1281.8^b	2081.2^b	3.3^b
Low (≤1.070)	male	44.9^a	128.9^a	621.9^a	1008.6^a	1494.4^a	2502.6^a	6.0^a
	female	44.5^a	124.1^b	564.2^b	884.4^b	1275.3^b	2067.1^b	2.8^b

[a,b] Means within a column by egg variable with different superscripts are significantly different (P≤.05).

* Means between egg sizes and specific gravities with asterisks are significantly different (P≤.05).

body weight early in life, but this influence declined with age.

Both male and female mortality was significantly higher from small versus large eggs (Table 1). Approximately 70% of the total mortality occurred within the first week of age for chicks from small eggs, with only 33% of the mortality for chicks from large eggs recorded during this period. Most of the early mortalty was attributed to dehydration, as excess urates were evident in the body cavity of posted birds.

Broilers from small eggs had a significantly better feed efficiency than broilers from large eggs at 49 days of age (Table 2). No differences were noted in feed efficiency between egg size groups at 28 days of age. Birds from 48 to 54 g eggs had a 6% better feed efficiency when compared with birds from 58 to 64 g eggs. The ability of broilers from small eggs to compensate for some of the early weight difference that existed between them and broilers from large eggs may partially explain the difference in feed efficiency between the egg size groups. Birds from small versus large eggs had a difference in body weight of 14% at 28 days of age that was reduced to 9% at 49 days of age.

Within the small egg group the lighter eggs hatched before the heavier eggs (Table 3). This agrees with

Table 2. Influence of egg size and egg specific gravity
on feed efficiency[1]

	Feed Efficiency (days of age)	
	28	49
Egg size		
Large (58-64g)	.680[a]	.518[a]
Small (48-54g)	.680[a]	.549[b]
Egg specific gravity		
High (≥1.080)	.637[a]	.529[a]
Low (≤1.070)	.621[a]	.529[a]

[a,b]Means within a column by egg group with different
superscripts are significantly different (P≤.05).
[1]Body weight/feed consumed.

Table 3. Infuence of egg size and egg specific gravity on mean egg weight and placement weight by hatch period

		Hatch Period						
		1		2		3		4
		Held	Removed	Held	Removed	Held	Removed	Removed
Exp 1								
Egg weight (g)	Large (58–64g)	61.4^a	61.4^a	61.5^a	61.5^a	61.5^a	61.5^a	61.7^a
	Small (48–54g)	49.7^{bc} *	49.6^c *	50.2^{abc} *	50.1^a *	50.7^a *	50.5^a *	50.2^{ab} *
Placement[1] weight (g)	Large (58–64g)	38.9^e	44.8^a	40.7^d	45.0^a	41.5^c	44.7^a	43.0^b
	Small (48–54g)	32.7^d *	35.7^b *	33.8^c *	36.5^a *	34.2^c *	36.5^a *	35.6^b *
Exp 2								
Egg weight (g)	High (≥1.080)	62.3^a	62.5^a	61.9^a	61.9^a	62.0^a	62.0^a	62.3^a
	Low (≤1.070)	63.4^a *	63.5^a *	63.1^a *	63.0^a *	63.2^a *	63.1^a *	62.8^a
Placement[1] weight (g)	High (≥1.080)	40.6^e	45.2^a	41.4^d	44.8^{ab}	41.9^d	44.5^b	43.4^c
	Low (≤1.070	42.1^e *	46.5^a *	42.8^d *	46.0^{ab} *	43.2^{cd} *	45.5^b *	43.6^c

[1]Placement weight represents hatch weight for the removed groups. Hatch weights between held and removed chicks within hatch periods were not significantly different.

[a,b,c,d,e]Means within a row with different superscripts are significantly different ($P\le.05$).

*Means with a column with an asterisk are significnatly different ($P\le.05$).

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findings by Williams et al. (1951) and McNaughton et al.
(1978) who reported that embryos from larger eggs take
longer to develop than embryos from smaller eggs. However,
egg size had no influence on hatch time within the large egg
group. Furthermore, it was observed that chicks from the
large egg group hatched approximately 6 hrs before chicks
from the small egg group. These findings are contrary to
the results from the above cited researchers. The
difference in hatching times for the two egg size groups may
have been influenced by the lower hatchability from small
eggs (57%) when compared with large eggs (72%).

Eggshell quality (Exp 2)

After one week of age, egg specific gravity had no effect
on chick body weight (Table 1). Chicks from eggs with low
specific gravity (≤1.070) were significantly heavier (3%) at
placement time and significantly lighter at 7 days of age
than birds from eggs with high specific gravity (≥1.080).
This difference can be partially attributed to the low group
having significantly heavier (2%) mean egg weights than the
high group (Table 3). However, by 7 days of age, birds from
the high specific gravity group had compensated for the
lower placement weights and were 3 g heavier than chicks
from eggs with poorer shells. Even though these weights
were significantly different at 7 days of age, they were

numerically small and probably not meaningful.

Eggshell quality had no influence on feed efficiency at 28 or 49 days of age (Table 2), or on mortality at 49 days of age (Table 1). It was postulated that a lower specific gravity would cause more severe dehydration in chicks and would result in increased chick mortality. However, this did not occur. Male broilers had a significantly higher 49 day cumulative mortality (5.7%) than females (3.0%) (Table 1).

Post-hatch placement time (Exp 1 and 2)

Main effects. Chick placement weights were significantly influenced by hatch periods (HP) and removal time from the hatcher (Table 3). In Exp 1, the weight of early hatched chicks (removed chicks in HP's 1,2 and 3) within the large egg group were 4% heavier at the time of hatch than late hatched chicks (HP 4). However, in the small egg group, removed chicks from HP's 1 and 4 were significantly lighter than from HP's 2 and 3. The lighter weights from early hatching chicks among smaller eggs in HP 1 may have resulted from the lower egg weight mean in that hatch period. In Exp 2, chicks from the low specific gravity group had significantly heavier placement weights than the high specific gravity group in HP's 1, 2 and 3 (Table 3). This significant difference in placement weight may be partially

due to the heavier (1 g) egg weights in the low specific
gravity group (Table 3). Removed chick weights at placement
time in Exp 2 were gradually reduced from HP 1 to HP 4.
This reduction in weight cannot be attributed to a
difference in egg weight, but may have been caused by
additional dehydration that occurred between HP's 1 and 4.
These results generally agree with those of Moran and
Reinhart (1980) who reported that early emerging poults were
heavier than late poults, irrespective of egg weight.

Body weight loss significantly increased as the time
between hatch and placement increased (Table 3). The weight
advantage of chicks from the early hatch periods (HP 1-3)
was lost when they were left in the hatcher and removed with
chicks from HP 4 (21 days, 12 hrs). In Exp 1, the mean
weight losses of chicks held in the hatcher versus removed
during HP's 1, 2 and 3 were 12.2, 9.3 and 7.2%,
respectively. Furthermore, chicks that were held in the
hatcher in Exp 2 for 15 to 28 hrs after hatch weighed 5.3 to
11.3% less than hatchmates removed during HP 1-3 (Table 3).
These severe losses in weight between hatch and removal from
the hatcher demonstrate the amount of dehydration that can
occur during this period. These results agree with previous
research which showed that chick weight was directly
correlated with the length of time chicks are held in the
hatcher after hatch (Williams et al., 1951; Fanguy et al.,

1980; Hager and Beane, 1983; Reinhart and Hurnik, 1984).

A reduction in body weight was noted in chicks held in the hatcher when compared with removed chicks through 49 days of age (Table 4). In Exp 1, chicks that were held in the hatcher were 5.4% (6 g) lighter at 7 days of age than chicks removed within seven hours after hatch. The difference in body weight for the two hatch removal regimes continued throughout the experiment with chicks removed early weighing 1.6% (31 g) more at 49 days of age. Larger differences were found between the hatch removal regimes in Exp 2, with removed chicks weighing 11.5% (14 g) and 2.1% (48 g) more than held chicks at 7 and 49 days of age, respectively. These findings are in agreement with those of several studies (Williams et al., 1951; Fanguy et al., 1980; Moran and Reinhart, 1980; Hager and Beane, 1983) which have reported heavier body weights from chicks removed from the hatcher soon after hatching versus chicks held in the machine until the end of the hatching cycle.

Generally, a depression in weight was observed among chickens held in the hatcher when compared with removed hatchmates in the first three HP's to 49 days of age (Tables 5 and 6). Body weight at 49 days of age was reduced from 1 to 2% in HP's 1 thru 3 when chicks were held in the hatcher from 14 to 32 hrs versus when removed within 7 hrs after

Table 4. Influence of chick placement time on body weights
          (first three hatch periods only)

Holding Treatment	Body Weights (g)				
	7 days	21 days	28 days	35 days	49 days
Exp 1					
Removed	117^a	538^a	858^a	1232^a	2001^a
Held	111^b	524^b	839^b	1212^b	1970^b
Exp 2					
Removed	136^a	611^a	964^a	1407^a	2323^a
Held	122^b	588^b	932^b	1372^b	2275^b

[a,b] Means within a column for each Exp displaying different
superscripts are significantly different ($P \leq .05$).

Table 5. Influence of hatch period and chick removal time on body weight, Exp 1

(days)	Treatment	Hatch Period			
		1	2	3	4
		───────────────── (g) ─────────────────			
7	Removed	118.8^a}*	115.9^{ab}	115.3^b}*	110.3^c
	Held	108.5^b}*	112.8^a	110.1^{ab}}*	
21	Removed	542.1^a}*	534.7^a	534.4^a}*	518.7^b
	Held	525.0^a}*	528.1^a	518.6^a}*	
28	Removed	860.1^a}*	852.6^a	856.8^a}*	832.5^b
	Held	840.9^a}*	843.1^a	833.1^a}*	
35	Removed	1238.9^a}*	1234.6^a	1219.1^{ab}	1207.0^b
	Held	1213.0^a}*	1219.3^a	1206.7^a	
49	Removed	1993.7^a	2013.2^a}*	1993.0^a	2003.1^a
	Held	1976.7^a	1978.3^a}*	1971.9^a	

^{a,b}Means within a row displaying different superscripts are significantly different $(P \le .05)$.

*Means within a column by age with an asterisk are significantly different $(P \le .05)$.

Table 6. Influence of hatch period and chick removal time on body weight, Exp 2

(days)	Treatment	Hatch Period			
		1	2	3	4
				(g)	
7	Removed	137.0^a }*	138.9^a }*	132.3^b }*	121.1^c
	Held	127.5^a }*	117.9^b }*	121.6^c }*	
21	Removed	615.1^a }*	614.2^a }*	604.0^a }*	574.9^b
	Held	600.3^a }*	582.2^b }*	581.3^b }*	
28	Removed	968.9^{ab}	971.0^a }*	952.2^b }*	921.7^c
	Held	959.8^a	912.1^b }*	923.8^b }*	
35	Removed	1408.7^a	1411.4^a }*	1400.6^a }*	1351.7^b
	Held	1404.4^a	1351.0^b }*	1360.2^b }*	
49	Removed	2310.7^a	2314.7^a }*	2343.7^a }*	2244.7^b
	Held	2323.0^a	2239.9^b }*	2260.6^b }*	

[a,b]Means within a row displaying different superscripts are significantly different ($P \leq .05$).

*Means within a column by age with an asterisk are significantly different ($P \leq .05$).

hatch (Exp 1). Furthermore, in Exp 2 a 3 to 4% reduction in body weight was noted at 49 days of age from late versus early removed birds in HP's 2 and 3. However, no weight differnce was noted among birds in the two removal times in HP 1 after 21 days of age. Initially it was postulated that chicks in the first group held in the hatcher would be the most severely stressed and would have depressed body weights. However, in Exp 2 these birds were able to compensate for the stressful conditions associated with extended holding in the hatcher by 28 days of age.

Chicks in HP 4 weighed significantly less than removed chicks from HP's 1 through 3 for the first 28 days in Exp 1 and through 49 days of age in Exp 2 (Tables 5 and 6). Chicks from the last hatch period weighed 3 and 4% less at 28 days of age in Exp's 1 and 2, respectively; and 3% less at 49 days of age in Exp 2. Even though these birds were removed from the hatcher soon after hatching, total incubation time was 8 hrs longer than for the other three removal groups which allowed more time for dehydration.

The length of time chicks were held in the hatcher after hatch had no significant influence on 28 or 49 day feed efficiency in Exp 1 or 2. Also, no significant effect was observed for 28 or 49 day feed efficiency between hatch periods. Hager and Beane (1983) reported similar results,

as they found that holding treatments and hatch periods had
no significant effect on 28 day cumulative feed efficiency.

Although numerical differences were noted, no significant
differences were found for mortality between holding
treatments in Exp 1 or 2.

Interactions. A significant hatch treatment X egg size
interaction was observed for body weight at 7, 14 and 49
days of age. The sexes were pooled for this analysis as no
hatch treatment X sex interaction was found. It appears in
Figure 1 that birds from large eggs were affected more by
extended holding time in the hatcher than birds from small
eggs. Chicks from large eggs that were held in the hatcher
more than 24 hrs (1H and 2H) weighed 3% less at 49 days of
age than hatchmates that were removed within 7 hrs after
hatch. However, in chicks from small eggs, the weight
difference between holding and removal treatments in HP's 1
and 2 was less than 1%. Chicks from small eggs are possibly
able to compensate for the depressive effects of delayed
placement on early body weight more rapidly than chicks from
large eggs. These findings agree with those of O'Neil
(1955) who reported that chicks hatching with the highest
percentage of original egg weight were heavier at 56 days of
age. In this study, chicks held in the hatcher from small
eggs maintained a higher percentage of their original egg

Figure 1. Influence of egg size and hatch periods
on body weight at 7 and 49 days of age,
Exp 1 (R= Removed, H= Held).

weight than held chicks from large eggs. Also, mortality was significantly greater in the small egg group which may have eliminated weaker chicks and lowered body weight variability between held and removed treatments (Table 1).

A significant hatch treatment X egg specific gravity interaction was observed in Exp 2. At 7, 28 and 49 days of age, body weight of chicks from eggs with high specific gravity were more severely affected in the second held hatch treatment (2H) than chicks from eggs with poor shells (Figure 2). Also, some inconsistencies in body weight were recorded among chickens from the two specific gravity groups for different hatch treatments. However, none of these differences can be logically explained by the treatments imposed in these studies.

All chickens in both experiments were raised using recommended husbandry practices. Possibly the added stress of poor flock management during the growing phase, which can occur commercially, in combination with delayed removal from the hatcher would further depress the performance of held versus removed chicks. Furthermore, chicks held in the hatcher for extended periods of time exhibited "delayed placement syndrome" which Fanguy et al. (1980) characterized as dehydration, early mortality and reduced weight gain. With the increased requirement for more specific body

Figure 2. Influence of egg specific gravity and
hatch periods on body weight at 7, 28
and 49 days of age, Exp 2 (R= Removed,
H= Held).

weights in the market place and the use of mechanical
eviscerating equipment during processing, flock uniformity
today is of major concern. The difference associated with
removing chicks once versus several times during a normal
hatching cycle (36 hrs) can amount to a greater variation in
body weights at market age. Also, it is important to note
that the prompt removal of chicks from the hatcher and
placement in growing pens can contribute to increased growth
and reduced mortality and morbidity.

Analyses of variance showing degrees of freedom, sum of
squares and levels of significants for variables used in
this experiment are presented in Appendix Tables 1, 2, 3, 4,
5, 6 and 7.

Summary

Although some inconsistencies were observed between hatch
periods, the results from both experiments indicate that
broiler body weights were significantly reduced by delayed
removal of chicks from the hatcher through 49 days of age.
In addition, egg size had a significant influence on broiler
body weights throughout the growing period. No meaningful
differences were noted in body weight or feed efficiency for
eggshell quality.

Part 2

Influence of two holding times in the hatcher on several physiological parameters associated with the immune system of chickens

Introduction

Adverse environmental conditions can be detrimental to the general health and thriftiness of newly hatched chicks. Also, chicks subjected to high levels of post-hatch stress may be more susceptible to infectious diseases. Stressful conditions may directly kill the chick or enhance the opportunity for microorganisms to infect and spread through the flock.

Cheville (1978) reported that inhibition of a primary immune response may occur when chicks are exposed to high environmental temperatures prior to an antigenic stimulus from a vaccine. The exposure of chicks to thermal stress has been reported to stimulate an increase in the secretion of adrenocorticotrophin (ACTH) (Thaxton, 1978). Previous work has shown that the secretion of ACTH caused the release of adrenocortical hormones (glucocorticoids) which elicited physiological changes in the bird (Resko et al., 1964). Glick (1967) and Siegel (1971) reported that adrenocorticoid secretion may cause decreased bursa and spleen weight, depressed blood protein level and elevated blood glucose level. Also, glucocorticoids were found to prompt suppression of both humoral and cellular immune responses (Thaxton et al., 1968; Sato and Glick, 1970).

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The objectives of this study were to evaluate the influence of two chick holding times in the hatcher and eggshell quality on several physiological parameters associated with the immune system. Also, the influence of two chick holding times in the hatcher in conjunction with a B_1, Newcastle disease virus (NDV) and Mycoplasma Gallisepticum (MG) challenge on respiratory infection and growth were studied.

Materials and Methods

Two experiments were conducted using commercial broiler chicks hatched during the first quarter of a normal hatching cycle. All chicks were removed from the hatcher, feather sexed, weighed and wingbanded at 20 days 6 hrs of incubation. One-half of the chicks were vaccinated for Marek's disease and held in chick boxes at 21° C for 6 hrs prior to being placed in growing pens (1.52 x 3.66 m). The remaining half were returned to the hatcher and held until 21 days, 12 hrs of incubation then reweighed, vaccinated for Marek's disease and held for 6 hrs in chick boxes at 21° C prior to placement. Twenty one days twelve hours after the eggs were set was considered zero days of age. All chicks were grown under commercial type light and temperature regimes and provided feed and water ad libitum until sacrificed.

Experiment 1 (Exp 1). One hundred and eighty chicks (90 removed and 90 held) were used. Ten chicks from each hatching treatment were weighed and sacrificed at 2, 4, 6, 8, 10, 14, 21, 28 and 35 days of age. The bursa of Fabricius was ectomized, weighed and expressed as relative weight [mg of organ/100 g of live body weight (BW)]. Prior to sacrificing, blood samples were collected by cardiac puncture at 2, 4, 6, 8, 10 and 14 days of age. Plasma samples were analyzed colorimeterically for glucose (Sigma kit #510). Additional blood samples were collected in heparinized microhematocrit tubes for the measurement of pack cell volume (PCV) and total plasma protein (TPP) levels. Total plasma protein was determined using a refractometer (American Optical Model 10400 TS) utilizing the protein scale described by Morgan et al., (1975).

Experiment 2 (Exp 2). A total of 864 commercial broiler chicks were used to study the influence of two hatcher holding periods and two eggshell qualities (egg specific gravity, ESG) on several physiological parameters. Egg specific gravities of ≤1.065 (poor shell quality) and ≥1.075 (good shell quality) were used. Forty-eight chicks from each treatment group (two ESG and two hatcher holding periods) were weighed and sacrificed at 1, 3, 5, 7, 9, 11, 14, 21 and 28 days of age. Bursa and spleen weights were

collected and expressed as described for bursa weight in Exp
1. Prior to sacrificing, blood samples were collected on 1,
3, 5, 7, 9 and 11 days of age for TPP and PCV
determinations. Plasma glucose levels and
heterophil/lymphocyte (H/L) ratios were determined from
blood samples at 1, 3 and 5 days of age. Plasma glucose,
TPP and PCV measurements were determined as described in Exp
1. A stained slide method was used to determine the number
of heterophils and lymphocytes present by examining a blood
smear prepared by slide centrifugation in a Larc Spinner
(Gross and Siegel, 1983). The smears were stained using
May-Grunwald-Giemsa stain. At least 60 cells per smear were
counted for each ratio. The heterophil/lymphocyte ratio was
determined by dividing the number of heterophils by the
number of lymphocytes. Heterophil/lymphocyte ratios from
blood smears collected at 1 day of age were not used in this
study because of inferior slide quality which was due to an
anticoagulant problem.

Twenty chicks from the two hatcher removal regimes were
randomly assigned to two batteries and were provided a
brooding temperature of 27 or 35°C. At one day of age all
chicks were aerosol challenged with a combination of B_1, NDV
and MG. Mortality, body weight gain (BWG) and air sac
lesion scores were measured at 28 days of age. Air sacs
were examined for lesions and were scored on a scale from 1

to 5, with 1 representing low infection and 5 severe

infection. This portion of the experiment was conducted in

cooperation with Dr. Gross, Professor, Virginia-Maryland

Regional College of Veterinary Medicine and is included in

this thesis because the data are critical to the

interpretation of the immune response.

<u>Statistical</u> <u>Analyses</u>. Individual body weights, relative

spleen and bursa weight, H/L, PCV, TPP and plasma glucose

were compared using analysis of variance to determine

significant differences in treatment means. When a

significance level of P ≤ 0.05 was obtained with the

analysis of variance, Duncan's multiple range test was used

to seperate mean differences within treatments (P ≤ 0.10 was

significance level for RBW). Analyses were made using the

following statistical model:

Experiment 1 $Y_{ij1} = u + T_i + S_j + (TS)_{ij} + e_{ij1}$

Experiment 2 $Y_{ijkl} = u + T_i + S_j + G_k + (TS)_{ij} + (TG)_{ik} + (SG)_{jk} + (TSG)_{ijk} + e_{ijkl}$

where i = 1,2 hatch treatments (T), j = 1,2 sex (S), k=1,2

specific gravity groups (G) and l = 1,2 . . . n individuals.

Relative bursa and spleen weights were calculated as a

percentage and were transformed to arc sin % prior to

analyses (Snedecor and Cochran, 1980).
Heterophil/lymphocytre ratics were transformed to the log
base 10 prior to analysis.

The effect of age on each dependent variable for each
treatment was determined using analysis of variance. The
statistical model used was: $Y_{ij} = u + A_i + e_{ij}$
where i = 1,2,3,4,5,6,7,8 and 9 ages (A) and j = 1,2 . . . n
individuals.

Mortality, BWG and air sac lesion scores were analyzed
using Fisher's exact test to determine significant
differences.

Results

Experiment 1

Chicks held in the hatcher 30 hrs post-hatch had
significantly reduced relative bursa weights through 8 days
of age when compared with hatchmates removed within 7 hrs
after hatching and placed 6 hrs after removal (Figure 1).
By 21 days of age, held chicks had compensated and had
significantly larger relative bursa weights (306 mg/100 g
BW) than removed birds (245 mg/100 g BW). Total plasma
protein and glucose were not significantly different between
post-hatch holding treatments. However, a significant
difference was found between the sexes for TPP levels and

Figure 1. Influence of two post-hatch holding times on bursa weights in broiler chicks. (Exp 1)

PCV, with females being higher in both parameters than males through 14 days of age. Also, PCV value were significantly higher in removed chicks (29%) than in held chicks (26%) at 3 days of age.

Experiment 2

Body weights. Body weight means are presented in Table 1. Chicks held in the hatcher for 30 hrs had significantly lower BW through 21 days of age when compared with removed hatchmates. Males had significantly higher mean BW than females from 3 through 28 days of age. Body weight means were not significantly affected by ESG groups at any of the ages.

Bursa and spleen weights. The influence of the two post-hatch holding times on relative bursa weight (RBW) is shown in Figure 2. Relative bursa weights were depressed among held chicks through 14 days of age when compared with removed chicks. Chicks held in the hatcher had a mean RBW that was 21.1% less than removed chicks at 1 day of age, but by 14 days of age the reduction was only 7.8%. By 21 days of age, no difference was noted in RBW between held and removed chicks. Relative bursa weight was significantly higher for males than for females from 1 through 14 days of age, with no significant differences noted thereafter (Figure 3).

Table 1. Influence of two post-hatch holding times on mean body weights for broiler chicks.

Treatment	Body weight (g)	
	Male	Female
Day 1		
Removed[1]	50.4^a }*	49.6^a }*
Held[2]	45.0^a	45.4^a
Day 3		
Removed	76.1^a }*	71.7^b }*
Held	70.5^a	59.8^b
Day 5		
Removed	105.6^a }*	101.8^b }*
Held	91.3^a	86.5^b
Day 7		
Removed	141.2^a }*	129.9^b }*
Held	121.9^a	114.3^b
Day 9		
Removed	192.8^a }*	180.3^b }*
Held	169.3^a	144.6^b
Day 11		
Removed	244.3^a }*	225.8^b }*
Held	217.7^a	193.7^b
Day 14		
Removed	344.7^a }*	328.5^b }*
Held	319.4^a	288.6^b
Day 21		
Removed	672.3^a }*	595.8^a }*
Held	634.9^a	548.7^a
Day 28		
Removed	1012.6^a	848.5^b
Held	1006.7^a	837.3^b

[1]Chicks were removed from hatcher at 20 day, 6 hr.

[2]Chicks were held in the hatcher 30 hrs post-hatch and removed at 21 days, 12 hrs.

[a,b]Means within a row and age displaying different superscripts are significantly different (P≤.05).

*Means within a column and age displaying an asterisk are significantly different (P≤.05).

Figure 2. Influence of two post-hatch holding times on bursa weights in broiler chicks. (Exp 2)

Figure 3.   Influence of sex on bursa weights in broiler
            chicks.

            *(P≤ .05)

Chicks removed early from the hatcher had significantly heavier RSW values at 1, 3, 9 and 11 days of age when compared with held chicks (Figure 4). Relative spleen weights were 29% less among the held chicks at 1 day of age and 6.3% less at 11 days of age. After 11 days of age no difference was noted in RSW between holding times. Also, no significant difference in RSW value was found between males and females.

Egg specific gravity had a significant influence on RBW at 1 day of age. Relative bursa weights from the lower ESG group were lighter than from the high ESG group. After 1 day of age, no differences in RBW was found between the ESG groups. Relative spleen weight was not significantly influenced by ESG.

Total plasma protein. A significant difference in total plasma protein was found between post-hatch holding times for males and females at 1 day of age (Table 2). Chicks held in the hatcher had a TPP level of 3.36 mg/100 g plasma compared to a lower level of 3.00 mg/100 g plasma for removed chicks. No differences between holding treatments were found for females after day 1 of age; however, males held in the hatcher had a significantly higher TPP level (3.47 mg/100 g plasma) than removed males (3.26 mg/100 g plasma) at 5 days of age.

Figure 4. Influence of two post-hatch holding times on spleen
weights in broiler chicks.

Table 2. Mean total plasma protein, hematocrit, and heterophil/lymphocyte ratio as influenced by two post-hatch holding times

| | Age (days) | | | | | |
| | 1 | | 3 | | 5 | |
Treatment	Male	Female	Male	Female	Male	Female
Total plasma protein (mg/dl)						
Removed[1]	2.84^a	3.15^a	3.12^a	3.52^a	3.26^a	3.52^a
Held[2]	3.29^b	3.43^b	3.27^a	3.31^a	3.47^b	3.41^a
Hematocrit (PCV)						
Removed	25.70^a	28.38^a	24.33^a	26.38^a	25.39^a	27.00^a
Held	29.04^b	28.88^a	23.10^b	25.52^a	25.34^a	27.04^a
Heterophil/lymphocyte ratio					*	
Removed	--	--	1.142^a	0.842^a	0.662^a	0.328^a
Held	--	--	1.441^b	1.742^b	1.141^a	0.825^b

[1]Chicks were removed from hatcher at 20 days, 6 hrs.
[2]Chicks were held in the hatcher 30 hrs post-hatch and removed at 21 days, 12 hrs.
a,bColumn within ages with different superscripts differ significantly ($P \leq .05$).
*Means between sexes displaying an asterisk are significantly different ($P \leq .05$).

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Total plasma protein levels increased through 5 days of age then sharply decreased at 7 days of age for both sexes (Figure 5). Female chicks had a higher TPP level through 3 days of age than males. From 7 through 11 days of age, TPP levels stabilized with no significant differences noted between males and females.

The lower ESG group had a significantly higher TPP level (3.27 mg/100 g plasma) than the high ESG group (3.10 mg/100 g plasma) at 1 day of age. Also, a significant sex X ESG interaction for TPP occurred at 5 days of age. Females in the high ESG group had a higher TPP level than in the low ESG group with the reverse relationship observed for males. Also, an interaction between ESG and chick removal times for TPP was found at 5 and 7 days of age. This was caused by higher TPP levels in the high ESG group as compared to the low ESG group in the held chicks with the opposite relationship occurring among removed chicks.

Hematocrit. Male chicks held in the hatcher for 30 hrs had a significantly elevated (12%) PCV at 1 day of age and a significantly reduced (5%) PCV at 3 days of age when compared with removed chicks (Table 2). Packed cell volume levels in female chicks did not differ between holding treatments at any age. Mean PCV decreased from 1 day of age to 3 days of age, then increased over the remaining time

Figure 5. Mean total plasma protein and hematocrit
for male and female broiler chicks.

periods (Figure 5). Hematocrit means for female chicks were significantly higher than for male chicks through 11 days of age.

Chicks from eggs with lower ESG had significantly higher PCV at 3 days of age. The low ESG group had a 25.6% PCV level compared to 24.0% PCV level for the higher ESG group. No significant differences were found between ESG groups at any of the other sample periods.

Heterophil/lymphocyte ratio. Results presented in Table 2 show the effect of chick holding times and sex on H/L. Heterophil/lymphocyte ratios were higher in held versus removed males and females at 3 days of age and in females at 5 days of age. No differences between sexes were recorded at 3 days of age, however, females had a significantly lower H/L than males at 5 days of age. Furthermore, an interaction involving holding treatments and sex for H/L was significant at 3 and 5 days of age. These results indicate that increased holding time had a greater effect on H/L in females than males.

Egg specific gravity had no influence on H/L at either age. Other first order interactions involving these traits were inconsistent and were not considered meaningful.

Plasma glucose. A significant ESG X chick removal time

interaction was noted for plasma glucose levels (Table 3). Glucose levels in the higher ESG group were 352 mg/dl from held chicks and 255 mg/dl from removed chicks at 1 day of age. However, in the low ESG group only a small non-significant difference was noted in glucose level between held and removed chicks. At day 5 of age, no difference in glucose level was found between chick holding treatments in the high ESG group; however, held chicks from the low ESG group had significantly higher glucose levels than removed chicks.

Mean plasma glucose was significantly different between ESG groups at 1 and 3 days of age (Table 3). The glucose levels of chicks from the low ESG group were 262 and 237 mg/dl and the levels in the high ESG groups were 305 and 256 mg/dl at 1 and 3 days of age, respectively. Males had a significantly higher glucose level (270 mg/dl) than females (251 mg/dl) at 5 days of age. No differences in this blood nutrient were recorded between sexes at the other sampling times.

Newcastle Disease Virus and Mycoplasma Gallisepticum challenge. Effects of aerosol challenge of B_1, NDV and MG on chicks held 30 hrs longer in the hatcher versus removed hatchmates are shown in Table 4. Although no significant differences were determined, mortality at 28 days of age was

Table 3. Mean plasma glucose levels (mg/dl) as influenced by egg specific gravity and post-hatch holding time

Treatment	Age (days)					
	1		3		5	
	Specific Gravity		Specific Gravity		Specific Gravity	
	1.065^3	1.075^3	1.065^3	1.075^3	1.065	1.075
Removed[1]	257^a	255^a	238^a	255^a	244^a	262^a
Held[2]	266^a	352^b	236^a	257^a	279^b	254^a

[1]Chicks were removed from hatcher at 20 days, 6 hrs.
[2]Chicks were held in the hatcher 30 hrs post-hatch and removed at 21 days, 12 hrs.
[3]Mean glucose levels between specific gravity groups differ significantly ($P \leq .05$).
[a,b]Columns within age and specific gravity with different superscripts differ significantly.

Table 4. Effect of a combination B_1, newcastle disease vaccine-mycoplasma gallisepticum aerosol challenge on chicks provided two post-hatch holding times

Treatment	n	Mortality (%)	Air Sac Lesion Scores (1-5)	Lesions >2 (%)	Body weight @28 days of age	Body Weight Gain[3] (g)
Removed[1]	20	0^a	1.10^a	5^a	550.5^a	116.0^a
Held[2]	20	20^a	3.20^b	60^b	515.9^a	101.1^b

[1]Chicks were removed from hatcher at 20 days, 6 hrs.
[2]Chicks were held in the hatcher 30 hrs post-hatch and removed at 21 days, 12 hrs.
[3]Between 3 and 4 weeks of age.

[a,b]Means within a column with different superscripts differ significantly.

numerically higher in the held (20%) when compared with
removed (0%) chicks. Air sac lesion scores and lesion
severity were significantly higher in held than in removed
chicks. Lesion scores were three fold higher and severity
was 12 fold higher for chicks exposed to the additional
stress of prolonged holding in the hatcher. Body weight
gain between 21 and 28 days of age was 14.7% higher in the
removed versus held birds. Though body weight at 28 days of
age was greater among removed birds, the difference was not
significant. Brooding temperature was found to have no
influence on mortality, air sac lesion scores or BWG.

Analyses of variance showing degrees of freedom, sums of
squares and levels of significance used in this experiment
are presented in Appendix Tables 8, 9, 10, 11, 12, 13 and
14.

Discussion

Early in the life of the chick, high environmental
temperature can affect the responsiveness of the immune
system, as well as overall performance. Thaxton et al.
(1968) and Subba Rao and Glick (1977) demonstrated that high
environmental temperature retarded development of humoral
immunity in chickens. One explanation for this
immunosuppression is that increased temperature stimulates
the hypothalamic -> hypophyseal -> adrenocortical axis

increasing the circulating levels of glucocorticoids (Thaxton, 1978). Although several steroids are secreted from the adrenals, the primary secretion in birds is corticosterone (Frankel, 1970). Siegel (1971) reported that glucocorticoids elicited changes such as lymphatic tissue involution, blood protein and glucose level changes, white blood cell number alterations, and depressed growth.

In the present study, holding chicks in the hatcher for 30 hrs post-hatch elicited physiological changes in several parameters when compared with removed hatchmates. Chicks held in the hatcher had depressed body, bursa and spleen weights. Furthermore, the development of the spleen and bursa appeared to be dependent on each other as the weight of both of these glands was depressed proportionally and for the same length of time. These findings agree with those of Glick (1967) who reported that failure of normal spleen development was correlated with the inhibition of bursa development. These changes in gland weight are possibly due to the release of hormones during the period chicks were exposed to high incubation temperatures. Edens and Siegel (1975) reported that significant changes occurred in plasma corticosterone levels of chicks exposed to high environmental temperatures. Also, the same researchers concluded that high plasma corticosterone responses may cause physiological changes during high temperatures. This

explanation is consistent with results from studies by Siegel (1961; 1962), as he reported that ACTH injections resulted in depressed bursa and spleen weights. Also, Greenman and Zarrow (1961) found depressed body weights in birds injected with corticosterone and cortisone. The results from the present study indicate that these target organs (bursa and spleen) are responsive at an early age. Also, previous studies by Glick et al. (1956) and Chang et al. (1957) reported that the function of the bursa of Fabricius plays an important role in antibody production in chickens. No explanation is provided for RBW differences between males and females.

Chicks held in the hatcher had higher TPP levels than removed hatchmates at 1 day of age. These findings are contrary to results reported by Siegel (1971), who found that exposure to high environmental temperatures caused a decrease in plasma protein levels. He postulated that these proteins were used as a source for glucose through the gluconeogenic pathway. The reason for this apparent contradiction is not clear; however, an increase in immunoglobulin synthesis caused by increased time in the hatcher may have increased the TPP levels. Sabistan and Ste. Rose (1976) reported that immunoglobulin turnover increased in rabbits exposed to stress. Differences in TPP levels found between sexes and ESG were inconsistent.

Hematocrits are a parameter used to indicate blood viscosity or dehydration within animals. In the present study, male chicks that were held in the hatcher had significantly higher hematocrits than removed chicks. These results are in agreement with those of Chamblee and Morgan (1983) who found an increase in PCV within 24 hrs after a chick is deprived of water. Unexpectedly, held versus removed females were not found to have different PCV levels. Hematocrits were at the lowest in both sexes at 3 days of age. This decrease may be attributed to consumption of water by the chicks which resulted in hemodilution. Differences in PCV between the sexes through 11 days of age cannot be explained.

Data from this study showed significant changes in H/L between chicks from the two holding treatments. Chicks held in the hatcher for a longer period of time had a higher H/L than their earlier removed hatchmates. Chancellor and Glick (1960) reported similar results with chicks exposed to high environmental temperatures as they found a marked increase in percentage of heterophils and decrease in percentage of lymphocytes. Previous studies by Glick (1958) and Gross and Siegel (1983) have shown that cortisone acetate and corticosterone given to chickens caused a decrease in the number of lymphocytes and an increase in the number of heterophils. Also, Gross and Siegel (1983) found that H/L

were higher in fasted chickens and chickens fed increased
levels of corticosterone.

Chicks that were held in the hatcher for 30 hrs had a
transient but significantly higher glucose level when
compared with hatchmates that were removed. Glucose levels
fluctuated; however, values remained within normal
physiological ranges (Hazelwood, 1976). The reason for
elevated glucose levels in held chicks is not clear, but may
have resulted from the release of hormones secreted by the
adrenal gland. Siegel and Beane (1961) found that a single
intramuscular injection of ACTH caused glucose levels to
increase within 12 to 24 hrs post-administration. Also,
Freeman and Manning (1977) showed that newly hatched chicks
had a 5% increase in plasma glucose level 4 hrs after an
ACTH injection. Chicks from the higher ESG group had higher
glucose levels than chicks from the lower ESG group. These
data suggest that holding time in the hatcher had a greater
influence on the glucose level of chicks from the higher ESG
group than chicks from the lower ESG group.

The combination of holding chicks in the hatcher for 30
hrs and a disease challenge (B_1, NDV and MG) caused an
increase in air sac lesion and severity scores when compared
with disease challenged removed birds. These responses are
consistent with those reported by Gross and Colmano (1969)

where stressed birds had a decreased resistance to viral infections. Also, chicks that were held in the hatcher had depressed BWG when compared with removed chicks between 21 and 28 days of age. Gross and Siegel (1982) found that when chicks were stressed early in life by either overheating or water deprivation they had lower body weights later in life. The early stress associated with holding chicks in the hatcher for a longer period of time may alter their immunity response and growth later in life.

These results suggest that holding chicks in the hatcher can be a stress that will alter their early immune response. The physiological changes which occurred in this study are similar to those reported when young chicks are temperature stressed or injected with ACTH. However, further studies should be performed to determine the significance of these physiological alterations on the immune system. Also, studies should be conducted to determine the economic aspects of the immunosuppressive effect of steroids on newly hatched chicks. This knowledge will allow for a better understanding of desired management programs for starting chicks.

Summary

Holding chicks in the hatcher for 30 hrs post-hatch resulted in depressed body weights, reduced bursa and spleen

weights, and increased H/L when compared with chicks removed soon after hatching. Hematocrits, plasma protein and glucose were not consistently changed by post-hatch holding time.

SUMMARY AND CONCLUSIONS

Two studies were conducted to determine the influence of egg size, eggshell quality and various holding times in the hatcher on the performance of broiler chickens. Furthermore, the influence of two hatcher removal schedules in conjunction with eggshell quality on several physiological parameters associated with the chick immune system were evaluated.

Egg size was found to influence body weight through 49 days of age, with birds from small eggs weighing significantly less than chicks from large eggs at each measurement period. However, eggshell quality, as measured by specific gravity (≤ 1.070 and ≥ 1.080) , was found to only influence body weight at placement time and 7 days of age.

Holding chicks in the hatcher for extended periods of time resulted in significantly reduced body weights throughout the growing period when compared with chicks that were removed within 7 hrs after emerging from the shell. Chicks that were held in the hatcher from 14 to 32 hrs had significantly lower placement weights than early removed chicks. This difference in body weight between the two removal groups continued through 49 days of age. The increased post-hatch holding time caused held chicks to be

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1.6% (Exp 1) and 2.1% (Exp 2) lighter than early removed chicks at 49 days of age.

Egg size was found to have a significant effect on feed efficiency at 49 days of age. Chicks from small eggs had a higher feed efficiency than chicks from large eggs at this age. Eggshell quality and hatch periods were found to have no influence on feed efficiency at 28 or 49 days of age.

Broiler mortality was significantly higher in birds from small eggs when compared with birds from large eggs. Also, males had a significantly higher mortality than females. No differences were found in mortality for eggshell quality or hatch periods.

The delayed removal of chicks from the hatcher was found to have an influence on several physiological parameters. Chicks that were held in the hatcher 30 hrs had significantly reduced relative bursa and spleen weights when compared with early removed chicks. Also, plasma protein and glucose levels were significantly reduced by the increased holding time at 1 day of age. Chicks that were removed early from the hatcher had a significantly lower hematocrit than held chicks at 1 day of age, indicating that removed chicks were less dehydrated than their held hatchmates. Heterophil/lymphocyte ratios were also influenced by removal times, as chicks held in the hatcher

had significantly higher H/L than chicks removed at 3 and 5 days of age.

Relative bursa weights were significantly lighter in females than males through 14 days of age. Total plasma protein and hematocrits were influenced by sex, with females having higher TPP levels through 5 days and higher hematocrits through 11 days of age than males. Differences in H/L between sexes were variable, but generally males had a significantly higher H/L than females. Eggshell quality was found to have no significant influence on any of these physiological parameters.

Results from these studies show that various post-hatch holding times have an influence on broiler growth and morbidity. Even though some of the birds from the hatch treatments were able to compensate for early weight depression by market age, flock uniformity and health status was reduced. Furthermore, egg size was found to have an influence on body weight, feed efficiency and mortality. Also, the time chicks are removed from the hatcher after hatch may play a greater role in early chick immune responses than was earlier realized. Even though the chick may be able to compensate for early depressions in body weight, it is doubtful they can compensate for early depressions in the immune response. This would be

especially true of young chicks exposed to disease organisms during a period when their immune responsiveness was low.

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APPENDIX

Appendix Table 1.   Analyses of Variance for Hatching Variables
(Exp 1)

Variable	Source of Variation	degrees of freedom	Anova SS
Egg weight	T(Treatment)	6	65.42**
	E(Egg size)	1	61500.98**
	T x E	6	46.66*
	Error	1977	6527.71
	Total	1990	69436.73
Placement weight	T	6	5931.09**
	E	1	27835.45**
	T x E	6	437.07**
	Error	1977	6943.53
	Total	1990	41923.82
Hatch weight	T	6	479.01**
	E	1	33712.57**
	T x E	6	159.18**
	Error	1977	7069.97
	Total	1990	42369.56
Held weight	T	2	616.59**
	E	1	9017.18**
	T x E	2	40.56**
	Error	818	2603.97
	Total	823	11997.54

*$P \leq .05$, **$P \leq .01$

Appendix Table 2. Analyses of Variance for Hatching Variables
                   (Exp 2)

Variable	Source of Variation	degrees of freedom	Anova SS
Egg weight	T(Treatment)	6	52.79 **
	S(Specific gravity)	1	455.52
	T x S	6	27.02
	Error	1795	16298.22
	Total	1808	16808.25
Placement weight	T	6	4151.60 **
	S	1	560.61 **
	T x S	6	109.04 *
	Error	1795	11927.00
	Total	1808	16769.65
Hatch weight	T	6	1393.68 **
	S	1	477.26 **
	T x S	6	87.25 *
	Error	1795	12295.34
	Total	1808	14176.94
Held weight	T	6	170.94 **
	S	1	350.70 **
	T x S	6	1.31
	Error	702	4267.72
	Total	707	4799.64

* P≤.05, ** P≤.01

Appendix Table 3. Analyses of Variance for Broiler Body Weight (Exp 1)

Source of Variation	7 days		14 days		21 days	
	D.F.	Anova SS	D.F.	Anova SS	D.F.	Anova SS
T(Hatch Treatment)	6	15747.70**	6	47402.36**	6	88765.35**
Held(H) vs Removed(R)[1]	1	7020.21**	1	19668.63**	1	24257.92*
1-H vs 1-R	1	9874.17**	1	21806.06**	1	27635.76**
2-H vs 2-R	1	957.35	1	4962.77**	1	4193.52*
3-H vs 3-R	1	2662.99**	1	10561.01**	1	24469.19
1-R vs 2-R	1	791.49	1	1732.22	1	5350.75
1-R vs 3-R	1	1135.09*	1	2213.62**	1	5653.74**
1-R vs 4-R	1	6695.30**	1	23311.94**	1	51124.08**
2-R vs 3-R	1	33.29	1	33.42	1	6.78*
2-R vs 4-R	1	2968.71**	1	12691.49**	1	24269.24*
3-R vs 4-R	1	2349.97**	1	11270.23**	1	23137.61
1-H vs 2-H	1	1750.57*	1	1447.69	1	947.01
1-H vs 3-H	1	232.37	1	0.04	1	3915.83
2-H vs 3-H	1	724.91	1	1505.89**	1	8902.74**
E(Egg size)	1	189593.80**	1	770536.18**	1	2043789.40**
S(Sex)	1	1322.91*	1	47953.45**	1	824536.67**
T x E	6	9287.18**	6	26689.77	6	39821.37
T x S	6	2983.88	6	6614.23	6	15528.92
E x S	1	391.18	1	142.33	1	3076.48
T x E x S	6	958.52	6	8406.25	6	38885.16
Error	1326	384004.56	1320	1705229.05	1314	4843062.22
Total	1353	607851.46	1347	2632802.25	1341	7993041.16

*$P \le .05$, **$P \le .01$

[1] Analyses includes first 3 hatch periods only

Appendix Table 3(cont'd). Analyses of Variance for Broiler Body Weight (Exp 2)

Source of Variation	28 days		35 days		42 days		49 days	
	D.F.	Anova SS	D.F.	Anova SS	D.F.	Anova SS	D.F.	Anova SS
T[1]	6	137911.97**	6	184331.37*	6	210978.77*	6	262576.64**
H vs R	1	41572.15*	1	45857.22*	1	91176.83*	1	203561.21
1-H vs 1-R	1	34638.62*	1	62498.86*	1	90900.07	1	26915.87*
2-H vs 2-R	1	8703.44	1	22400.21	1	50062.04	1	116833.64
3-H vs 3-R	1	51729.97**	1	14826.46	1	3704.56	1	42595.56
1-R vs 2-R	1	5487.95	1	1837.79	1	2902.39	1	36517.25
1-R vs 3-R	1	1072.57	1	37357.79**	1	40937.84	1	48.71
1-R vs 4-R	1	70796.65**	1	94824.02	1	31387.26	1	8086.73
2-R vs 3-R	1	1700.85	1	23184.00*	1	66645.90	1	39229.16
2-R vs 4-R	1	38220.58**	1	72030.25	1	53958.73	1	9649.51
3-R vs 4-R	1	55011.33**	1	13580.73	1	555.85	1	9396.37
1-H vs 2-H	1	481.51	1	3693.23	1	17990.91	1	235.82
1-H vs 3-H	1	4903.75	1	3850.42	1	1669.89	1	2149.82
2-H vs 3-H	1	8639.40**	1	15435.65**	1	9039.22**	1	3880.03**
E	1	4548284.05**	1	7242405.17**	1	9600700.75**	1	10662106.76**
S	1	4230935.62**	1	13470124.53**	1	28382355.88**	1	53642680.55*
T x E	6	75327.41	6	156041.10	6	168505.68	6	422024.81
T x S	6	30669.13	6	56522.91*	6	86734.64	6	157097.64
E x S	1	26026.12	1	56576.13	1	48375.29	1	13881.78
T x E x S	6	89983.68	6	145166.32	6	258195.87	6	314473.02
Error	1311	10031739.34	1307	17212774.31	1305	29018575.50	1300	35912225.87
Total	1338	19497593.03	1334	39203304.94	1332	68976516.68	1327	103146642.2

*$P \leq .05$, **$P \leq .01$

[1] Analyses includes first 3 hatch periods only

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Appendix Table 4. Analyses of Variance for Broiler Body Weight (Exp 2)

Source of Variation	7 days		14 days		21 days	
	D.F.	Anova SS	D.F.	Anova SS	D.F.	Anova SS
T(Hatch Treatment)	6	78065.41**	6	166154.75**	6	305289.66**
Held(H) vs Removed(R)[1]	1	54101.03**	1	91044.63**	1	151415.90*
1-H vs 1-R	1	8284.45**	1	14534.72**	1	20049.59**
2-H vs 2-R	1	42734.16**	1	81225.79**	1	96762.33**
3-H vs 3-R	1	11199.57	1	13827.33	1	49588.93
1-R vs 2-R	1	362.39**	1	656.04	1	66.34
1-R vs 3-R	1	2000.79**	1	5131.03**	1	11468.28**
1-R vs 4-R	1	23298.66**	1	70237.61**	1	148400.97
2-R vs 3-R	1	4172.31**	1	9682.51**	1	10024.46**
2-R vs 4-R	1	30201.80**	1	86306.60**	1	145637.36**
3-R vs 4-R	1	11998.77**	1	38589.33**	1	79975.90**
1-H vs 2-H	1	8904.53**	1	18578.10	1	30391.75**
1-H vs 3-H	1	3363.53*	1	4483.13	1	34115.81
2-H vs 3-H	1	1361.92**	1	4921.50	1	84.85
E(Egg specific gravity)	1	2209.39**	1	1732.46**	1	11563.90**
S(Sex)	1	5309.41**	1	176508.50**	1	1143685.38**
T x E	6	12561.83	6	46435.20	6	37491.91
T x S	6	1534.66	6	8160.12	6	17919.01
E x S	1	16.88	1	297.02	1	448.83
T x E x S	6	879.14	6	5501.23	6	9922.51
Error	1317	383152.35	1312	1804991.53	1304	4583778.40
Total	1344	483425.88	1339	2207568.14	1331	6094538.21

*$P \leq .05$, **$P \leq .01$

[1] Analyses includes first 3 hatch periods only

Appendix Table 4(cont'd). Analyses of Variance for Broiler Body Weight (Exp 2)

Source of Variation	28 days D.F.	Anova SS	35 days D.F.	Anova SS	42 days D.F.	Anova SS	49 days D.F.	Anova SS
T	6	670612.82**	6	882082.63**	6	1309815.81**	6	1913641.38**
H vs R[1]	1	289613.21**	1	341961.42**	1	343832.51**	1	646979.46**
1-H vs 1-R	1	7516.30	1	1646.13	1	3817.88	1	13680.28
2-H vs 2-R	1	326492.75**	1	340888.48**	1	270430.86**	1	516452.06**
3-H vs 3-R	1	76818.75	1	153817.55	1	317814.12	1	642006.03
1-R vs 2-R	1	416.99	1	703.42	1	3969.47	1	1462.83
1-R vs 3-R	1	25715.82	1	5999.94	1	1014.43	1	97987.01
1-R vs 4-R	1	205172.39**	1	296702.20	1	470617.46	1	386980.01
2-R vs 3-R	1	33388.47*	1	11016.36	1	9159.89	1	77039.87
2-R vs 4-R	1	228809.77**	1	332104.32**	1	396372.10**	1	444685.74**
3-R vs 4-R	1	87921.50**	1	223148.95**	1	523770.10**	1	891026.13**
1-H vs 2-H	1	211758.20**	1	264061.57**	1	412221.98**	1	636459.77**
1-H vs 3-H	1	121506.36	1	182184.95	1	349619.30	1	360976.24
2-H vs 3-H	1	12850.87	1	7873.01	1	2790.07	1	39976.07
E	1	6610.08	1	930.31	1	13678.94**	1	28244.63**
S	1	4799987.63**	1	14471127.99**	1	32219456.86**	1	60177798.73**
T x E	6	187062.61	6	388197.99	6	458772.82	1	730943.40
T x S	6	12888.06	6	37357.97	6	55187.97	6	288861.85
E x S	1	3060.45	1	21906.01	1	14068.11	1	6902.99
T x E x S	6	44370.63	6	102678.85	6	90931.59	6	183928.96
Error	1295	10866671.84	1289	19420683.14	1282	29830475.43	1268	42726959.64
Total	1322	16563567.56	1316	35329869.31	1309	64047066.87	1295	106424899.0

*$P \leq .05$, **$P \leq .01$

[1] Analyses includes first 3 hatch periods only

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Appendix Table 5. Analyse of Variance for Feed Efficiency at 28
and 49 days of age (Exp] and 2)

Day	Source of Variation	degrees of freedom	Anova SS
Exp 1			
28	T(Treatment)	6	0.0055
	E(Egg size)	1	0.0001
	R(Rep)	1	0.0000
	T x E	6	0.0070
	Error	13	0.1010
	Total	27	0.1137
49	T	6	0.0091 *
	E	1	0.0890
	R	1	0.0003
	T x E	6	0.0114
	Error	13	0.0467
	Total	27	0.1563
Exp 2			
28	T	6	0.0258
	S(Specific gravity)	1	0.0066
	R	1	0.0057
	T x S	6	0.0433
	Error	12	0.1060
	Total	26	0.1939
49	T	6	0.0086
	S	1	0.0000
	R	1	0.0005
	T x S	6	0.0091
	Error	12	0.0193
	Total	26	0.0376

*$P \leq .05$

Appendix Table 6. Analyses of Variance for Broiler Mortality
                   (Exp 1)

Day	Source of Variation	degrees of freedom	Anova SS
7	T(Treatment)	6	0.0264**
	E(Egg size)	1	0.0354
	S(Sex)	1	0.0001
	T x E	6	0.0204
	T x S	6	0.0085
	E x S	1	0.0006
	T x E x S	6	0.0029
	Error	28	0.1276
	Total	55	0.2218
28	T	6	0.0257**
	E	1	0.0568
	S	1	0.0003
	T x E	6	0.0206
	T x S	6	0.0084
	E x S	1	0.0017
	T x E x S	6	0.0036
	Error	28	0.1493
	Total	55	0.2665
49	T	6	0.0295**
	E	1	0.0500
	S	1	0.0032
	T x E	6	0.0237
	T x S	6	0.0089
	E x S	1	0.0023
	T x E x S	6	0.0072
	Error	28	0.1371
	Total	55	0.2618

**$P \leq .01$

Appendix Table 7. Analyses of Variance for Broiler Mortality
                   (Exp 2)

Day	Source of Variation	degrees of freedom	Anova SS
7	T(Treatment)	6	0.0024
	E(Specific gravity)	1	0.0004
	S(Sex)	1	0.0000
	T x E	6	0.0021
	T x S	6	0.0012
	E x S	1	0.0000
	T x E x S	6	0.0008
	Error	28	0.0092
	Total	55	0.0161
28	T	6	0.0066
	E	1	0.0014
	S	1	0.0011
	T x E	6	0.0084
	T x S	6	0.0193**
	E x S	1	0.0000
	T x E x S	6	0.0056
	Error	28	0.0237
	Total	55	0.0662
49	T	6	0.0081
	E	1	0.0000
	S	1	0.0098*
	T x E	6	0.0133
	T x S	6	0.0160
	E x S	1	0.0005
	T x E x S	6	0.0117
	Error	28	0.0497
	Total	55	0.1093

*P≤.05, **P≤.01

Appendix Table 8. Analyses of Variance for Physiological Parameters
                   (Exp 1)

Variable	Source of Variation	degrees of freedom	Anova SS
Bursa weight	T(Treatment)	1	0.0000**
(Arcbursa)	A(Age)	8	0.0064
	S(Sex)	1	0.0000*
	T x A	8	0.0006
	T x S	1	0.0000
	A x S	8	0.0003
	T x A x S	8	0.0004
	Error	153	0.0054
	Total	188	0.0137
Bursa weight[1]	T	1	0.0001*
(Arcbursa)	A	3	0.0004**
	S	1	0.0000
	T x A	3	0.0000
	T x S	1	0.0000
	A x S	3	0.0000
	T x A x S	3	0.0001
	Error	64	0.0013
	Total	79	0.0020
Total Plasma	T	1	0.2681**
Protein	A	5	3.3516**
	S	1	0.5939
	T x A	5	0.4775
	T x S	1	0.2633
	A x S	5	0.5973
	T x A x S	5	0.6178
	Error	95	7.8705
	Total	118	14.6187

[1]Includes only the first 4 sample days(2, 4, 6 and 8)
*$P \leq .05$, **$P \leq .01$

Appendix Table 8(cont'd). Analyses of Variance for Physiological
                           Parameters (Exp 1)

Variable	Source of Variation	degrees of freedom	Anova SS
Hematocrit	T	1	6.1997**
	A	5	211.1283**
	S	1	46.8800
	T x A	5	38.3656
	T x S	1	1.2436
	A x S	5	50.0349
	T x A x S	5	22.6894
	Error	96	522.0887
	Total	119	878.7917
Glucose	T	1	413.2424**
	A	4	31860.0087
	S	1	1.6265
	T x A	4	2206.9964
	T x S	1	1668.4782
	A x S	4	6551.3099
	T x A x S	4	5784.0761
	Error	78	62384.7143
	Total	97	116541.1939

*$P \leq .05$, **$P \leq .01$

Appendix Table 9.   Analyses of Variance for Bursa Weight by Age
                     (Arcbursa), Exp 2

Age	Source of Variation	degrees of freedom	Anova SS
1	T(Treatment)	1	0.00027**
	E(Specific gravity)	1	0.00008*
	S(Sex)	1	0.00012**
	T x E	1	0.00006
	T x S	1	0.00005
	E x S	1	0.00000
	T x E x S	1	0.00000
	Error	90	0.00149
	Total	97	0.00205
3	T	1	0.00005 +
	E	1	0.00003
	S	1	0.00007 *
	T x E	1	0.00000
	T x S	1	0.00006 *
	E x S	1	0.00001
	T x E x S	1	0.00005 *
	Error	88	0.00115
	Total	95	0.00142
5	T	1	0.00001
	E	1	0.00006
	S	1	0.00013 *
	T x E	1	0.00002
	T x S	1	0.00002
	E x S	1	0.00008
	T x E x S	1	0.00003
	Error	88	0.00195
	Total	95	0.00229
7	T	1	0.00006
	E	1	0.00007
	S	1	0.00030**
	T x E	1	0.00004
	T x S	1	0.00002
	E x S	1	0.00005
	T x E x S	1	0.00009
	Error	88	0.00310
	Total	95	0.00377

+P≤.10, *P≤.05, **P≤ .01

Appendix Table 9(cont'd). Analyses of Variance for Bursa Weight by Age (Arcbursa), Exp 2

Age	Source of Variation	degrees of freedom	Anova SS
9	T	1	0.00010 +
	E	1	0.00000 **
	S	1	0.00022
	T x E	1	0.00008
	T x S	1	0.00001
	E x S	1	0.00004
	T x E x S	1	0.00002
	Error	89	0.00283
	Total	96	0.00330
11	T	1	0.00003
	E	1	0.00000 **
	S	1	0.00065
	T x E	1	0.00001
	T x S	1	0.00000
	E x S	1	0.00002
	T x E x S	1	0.00000
	Error	88	0.00254
	Total	95	0.00327
14	T	1	0.00010 +
	E	1	·0.00000 *
	S	1	0.00023
	T x E	1	0.00002
	T x S	1	0.00003
	E x S	1	0.00004
	T x E x S	1	0.00001
	Error	88	0.00322
	Total	95	0.00367
21	T	1	0.00001
	E	1	0.00000
	S	1	0.00015
	T x E	1	0.00000
	T x S	1	0.00006
	E x S	1	0.00010
	T x E x S	1	0.00006
	Error	85	0.00515
	Total	92	0.00554

$^+$P≤.10, *P≤.05, **P≤.01

Appendix Table 9(cont'd). Analyses of Variance for Bursa Weight by Age (Arcbursa), Exp 2

Age	Source of Variation	degrees of freedom	Anova SS
28	T	1	0.00001
	E	1	0.00002
	S	1	0.00006
	T x E	1	0.00007
	T x S	1	0.00000
	E x S	1	0.00001
	T x E x S	1	0.00001
	Error	78	0.00266
	Total	85	0.00308

Appendix Table 10. Analyses of Variance for Spleen Weight by Age
(Arcspleen), Exp 2

Age	Source of Variation	degrees of freedom	Anova SS
1	T(Treatment)	1	0.00025**
	E(Specific gravity)	1	0.00001
	S(Sex)	1	0.00000
	T x E	1	0.00003
	T x S	1	0.00001
	E x S	1	0.00000
	T x E x S	1	0.00000
	Error	90	0.00074
	Total	97	0.00104
3	T	1	0.00011**
	E	1	0.00000
	S	1	0.00001
	T x E	1	0.00000
	T x S	1	0.00000
	E x S	1	0.00008**
	T x E x S	1	0.00002
	Error	88	0.00068
	Total	95	0.00091
5	T	1	0.00000
	E	1	0.00000
	S	1	0.00000
	T x E	1	0.00002
	T x S	1	0.00001
	E x S	1	0.00000
	T x E x S	1	0.00002
	Error	88	0.00112
	Total	95	0.00117
7	T	1	0.00002
	E	1	0.00000
	S	1	0.00001
	T x E	1	0.00004
	T x S	1	0.00008
	E x S	1	0.00000
	T x E x S	1	0.00000
	Error	88	0.00177
	Total	95	0.00191

**$P \leq .01$

Appendix Table 10(cont'd). Analyses of Variance for Spleen Weight by Age (Arcspleen), Exp 2

Age	Source of Variation	degrees of freedom	Anova SS
9	T	1	0.00009 *
	E	1	0.00000
	S	1	0.00004
	T x E	1	0.00002
	T x S	1	0.00000
	E x S	1	0.00000
	T x E x S	1	0.00002
	Error	89	0.00144
	Total	96	0.00163
11	T	1	0.00007 *
	E	1	0.00000
	S	1	0.00000
	T x E	1	0.00002
	T x S	1	0.00012 **
	E x S	1	0.00000
	T x E x S	1	0.00001
	Error	88	0.00138
	Total	95	0.00159
14	T	1	0.00003
	E	1	0.00003
	S	1	0.00003
	T x E	1	0.00002
	T x S	1	0.00000
	E x S	1	0.00003
	T x E x S	1	0.00002
	Error	88	0.00131
	Total	95	0.00148
21	T	1	0.00002
	E	1	0.00002
	S	1	0.00001
	T x E	1	0.00000
	T x S	1	0.00000
	E x S	1	0.00000
	T x E x S	1	0.00002
	Error	85	0.00199
	Total	92	0.00207

* $P \leq .05$, ** $P \leq .01$

Appendix Table 10(cont'd). Analyses of Variance for Spleen Weight
by Age (Arcspleen), Exp 2

Age	Source of Variation	degrees of freedom	Anova SS
28	T	1	0.00011
	E	1	0.00001
	S	1	0.00012
	T x E	1	0.00001
	T x S	1	0.00006
	E x S	1	0.00011
	T x E x S	1	0.00001
	Error	78	0.00368
	Total	85	0.00402

Appendix Table 11.   Analyses of Variance for Total Plasma Protein
                     by Age, Exp 2

Age	Source of Variation	degrees of freedom	Anova SS
1	T(Treatment)	1	3.2364**
	E(Specific gravity)	1	0.6882*
	S(Sex)	1	1.1418**
	T x E	1	0.1722
	T x S	1	0.1895
	E x S	1	0.0011
	T x E x S	1	0.0049
	Error	88	10.6653
	Total	95	15.8833
3	T	1	0.0006
	E	1	0.0185
	S	1	0.4476*
	T x E	1	0.1688
	T x S	1	0.1745
	E x S	1	0.0111
	T x E x S	1	0.0771
	Error	66	6.0490
	Total	73	8.6128
5	T	1	0.0066
	E	1	0.0005
	S	1	0.1722
	T x E	1	0.4821*
	T x S	1	0.7383**
	E x S	1	1.3275**
	T x E x S	1	0.1956
	Error	88	8.7015
	Total	95	11.5991
7	T	1	0.0059
	E	1	0.1611
	S	1	0.0355
	T x E	1	1.3816**
	T x S	1	0.3662
	E x S	1	0.3007
	T x E x S	1	0.0001
	Error	88	10.4784
	Total	95	12.4618

*P≤.05, **P≤.01

Appendix Table 11(cont'd). Analyses of Variance for Total Plasma
Protein by Age, Exp 2

Age	Source of Variation	degrees of freedom	Anova SS
9	T	1	0.0784
	E	1	0.2071
	S	1	0.0092
	T x E	1	0.0030
	T x S	1	0.0020
	E x S	1	0.0050
	T x E x S	1	0.0008
	Error	89	8.6203
	Total	96	8.9199
11	T	1	0.0190
	E	1	0.0167
	S	1	0.0136
	T x E	1	0.0087
	T x S	1	0.1594
	E x S	1	0.0642
	T x E x S	1	0.0013
	Error	86	4.0816
	Total	93	4.3543

Appendix Table 12. Analyses of Variance for Hematocrit by Age,
Exp 2

Age	Source of Variation	degrees of freedom	Anova SS
1	T(Treatment)	1	89.7121**
	E(Specific gravity)	1	0.0624
	S(Sex)	1	38.7415*
	T x E	1	0.0836
	T x S	1	49.0642*
	E x S	1	3.5392
	T x E x S	1	0.0791
	Error	89	635.8883
	Total	96	810.6237
3	T	1	26.5674*
	E	1	56.0483**
	S	1	115.5574**
	T x E	1	9.5844
	T x S	1	0.7216
	E x S	1	24.3077*
	T x E x S	1	5.4457
	Error	87	437.1579
	Total	94	682.0895
5	T	1	0.4228
	E	1	0.1583
	S	1	68.4820**
	T x E	1	13.8444
	T x S	1	0.4314
	E x S	1	0.3801
	T x E x S	1	29.3024*
	Error	88	539.2942
	Total	95	650.9349
7	T	1	14.0708
	E	1	5.4974
	S	1	39.5440*
	T x E	1	8.5523
	T x S	1	0.0795
	E x S	1	3.3371
	T x E x S	1	5.4934
	Error	88	610.0321
	Total	95	684.8724

*$P \leq .05$, **$P \leq .01$

Appendix Table 12(cont'd). Analyses of Variance for Hematocrit by
                             Age, Exp 2

Age	Source of Variation	degrees of freedom	Anova SS
9	T	1	2.2202
	E	1	0.7773
	S	1	28.8976 *
	T x E	1	1.9360
	T x S	1	1.9857
	E x S	1	0.4617
	T x E x S	1	2.6063
	Error	89	438.3333
	Total	96	478.4588
11	T	1	4.9111
	E	1	2.4302
	S	1	13.7848 *
	T x E	1	5.8235
	T x S	1	4.5793
	E x S	1	0.1857
	T x E x S	1	5.6444
	Error	86	278.0243
	Total	93	312.9787

*$P \leq .05$

Appendix Table 13. Analyses of Variance for Heterophil/
Lymphocyte Ratio by Age(Log_{10}), Exp 2

Age	Source of Variation	degrees of freedom	Anova SS
3	T(Treatment)	1	1.2989**
	E(Specific gravity)	1	0.0528
	S(Sex)	1	0.0996
	T x E	1	0.1775 *
	T x S	1	0.3136 *
	E x S	1	0.2989
	T x E x S	1	0.0101
	Error	87	4.7061
	Total	94	6.9489
5	T	1	1.6257**
	E	1	0.3403 *
	S	1	0.7009
	T x E	1	0.3332
	T x S	1	0.4929
	E x S	1	0.3096
	T x E x S	1	0.0020
	Error	87	10.2184
	Total	94	14.2612

*$P \leq .05$. **$P \leq .01$

Appendix Table 14. Analyses of Variance for Plasma Glucose by
Age, Exp 2

Age	Source of Variation	degrees of freedom	Anova SS
1	T(Treatment)	1	69572.51**
	E(Specific gravity)	1	42479.27**
	S(Sex)	1	4197.48
	T x E	1	46454.58**
	T x S	1	2978.26
	E x S	1	1960.19
	T x E x S	1	79.85
	Error	90	487579.67
	Total	97	661377.63
3	T	1	11.84
	E	1	9522.28**
	S	1	1032.05
	T x E	1	37.09
	T x S	1	3193.79
	E x S	1	738.26
	T x E x S	1	676.65
	Error	88	73331.20
	Total	95	88135.63
5	T	1	4769.16
	E	1	68.79
	S	1	6551.95*
	T x E	1	9056.23
	T x S	1	19.36
	E x S	1	188.06
	T x E x S	1	167.91
	Error	88	145156.14
	Total	95	167770.96

*$P \leq .05$, **$P \leq .01$

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INFLUENCE OF EGG SIZE, EGGSHELL QUALITY, AND HATCH AND PLACEMENT TIMES ON THE PERFORMANCE OF BROILER CHICKENS

by

CRAIG LAYNE WYATT

(ABSTRACT)

Small (48-54 g) and large (58-64 g) or low (≤1.070) and high specific gravity (≥1.080) eggs from broiler breeder flocks were used. The hatching sequence was divided into four periods with 25% of the chicks being hatched in each period. One-half of each group was weighed and returned to the hatcher and the remainder were placed in floor pens. Hatch-held chicks were removed at 21 days, 12 hrs, weighed and placed in floor pens. Effect of two post-hatch holding times (0 and 30 hrs) on bursa and spleen weights were measured through 35 days of age. Hematocrits, heterophil/lymphocyte ratios, plasma proteins and glucose were measured through 11 days of age.

Broilers from large vs small eggs were 10% heavier and had lower mortailty at 49 days of age. Chicks that were held for extended time in the hatcher had lower body weights throughout the growing period. Held chicks had 1.6% (Exp 1) and 2.1% (Exp 2) lighter body weights at 49 days of age than

removed chicks. No effect on body weight was observed for egg specific gravity.

Relative bursa and spleen weights were lower in held chicks through 14 days of age. Total plasma protein (TPP) and glucose levels were higher in the held chicks at 1, but lower at 3 days of age. Heterophil/lymphocyte ratios were higher in held females at 3 and 5 days and males at 3 days of age. Females had higher TPP levels through 5 days and hematocrits through 11 days of age.