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Title: Genetic Response to Divergent Selection for Blood Cholesterol Levels in Japanese Quail (Coturnix coturnix japonica)

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Cholesterol, $C_{27}H_{45}OH$, as a precursor of steroid hormones, and the genetic implications of a divergent selection program for blood cholesterol levels provided the purpose of this study.

The initial selection of Japanese Quail for the development of high and low blood cholesterol lines was from a randomly mated population of Japanese Quail. This study was conducted through four generations of offspring with no external stimuli introduced to any of the lines. After four generations of selection, lines were developed that differed significantly in their respective blood cholesterol levels. Production, fertility, hatch of fertile eggs, mortality and body weight were not found to be significantly different between the high and the low blood cholesterol lines. It was found that cholesterol level was negatively and significantly correlated with body weight.

The results of this study indicate that one of the possible uses of blood cholesterol levels would be as one of several traits used in an index selection program for the improvement of a particular line of birds.

Genetic Response to Divergent Selection for Blood Cholesterol Levels in Japanese Quail (*Coturnix coturnix japonica*)

by

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INTRODUCTION

Cholesterol, $C_{27}H_{45}OH$, is the most abundant sterol present in vertebrates and is synthesized *in vivo* from acetyl CoA as well as derived from several food sources. There are several external factors that affect the cholesterol level present in the blood: fasting, diets containing saturated or polyunsaturated fats, diets containing pure cholesterol, artificial illumination, the injection of adrenocorticotrophin hormone (ACTH), breeding season and age.

Blood cholesterol levels are also influenced by genetics and respond to selection. Research involving the genetic selection for high and/or low blood cholesterol levels in the avian species has yielded various results from various researchers. Heritability estimates for blood cholesterol levels in White Leghorns average 0.25 (Wilcox *et al.*, 1963) to 0.30 (Cherms *et al.*, 1960).

The relationship between blood cholesterol level and yolk cholesterol level is as yet unknown. Some studies have shown changes in the two to be parallel while other studies have shown no relationship (Washburn, 1975).

This study was undertaken to determine the response to a genetic selection program for blood cholesterol levels in Japanese Quail (*Coturnix coturnix japonica*); the associated heritabilities of blood cholesterol levels and the reproductive correlations. Observations of fertility, hatch of fertile eggs and of all eggs, egg production and

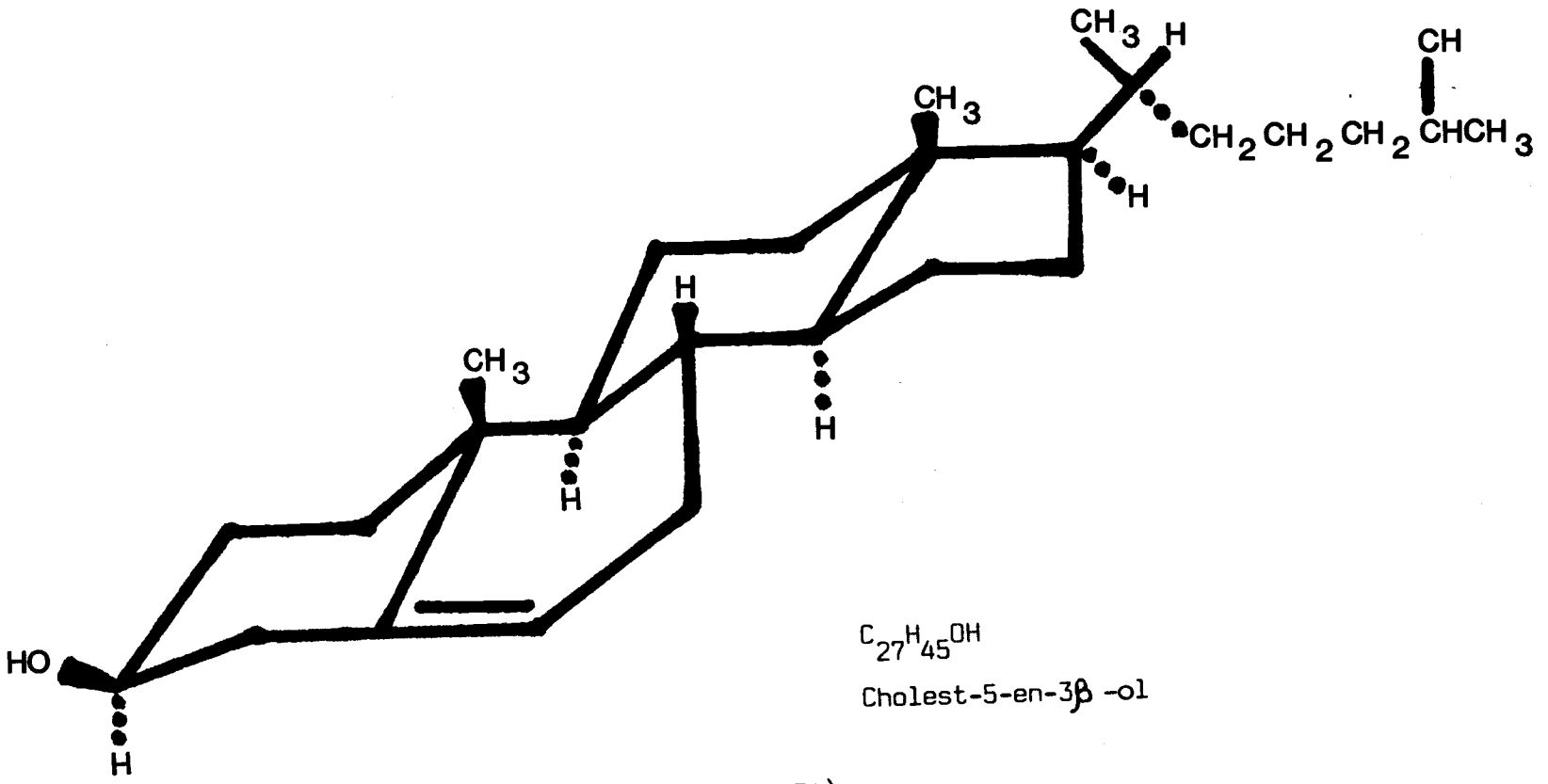
body weight were made so as to correlate them with blood cholesterol levels. This study involved no external stimuli to the production of blood cholesterol and is based solely on genetic selection.

REVIEW OF LITERATURE

Cholesterol - general.

Cholesterol is a pearly, fatlike monatomic alcohol with the composition of $C_{27}H_{45}OH$ (Dorland, 1974). It was recognized as a distinct chemical substance in 1816 and its structure was established by 1932. It is insoluble in water, soluble in many organic solvents and its crystals are birefringent. Cholesterol is the most abundant sterol in vertebrates and is present in the blood, liver, kidneys, bile, skin, sebum, nervous system, fat, muscle, connective tissue, arteries, intestine, glands, milk, faeces and distributed subcellularly. Cholesterol is also present in invertebrates where it participates in membrane formation and it serves as a precursor of moulting hormones in insects. Cholesterol is also present in green plants, some red algae and a few species of fungi (Myant, 1981).

The 27 carbon atoms of cholesterol are derived from acetyl CoA (Kice and Marvell, 1974). Eight of the carbon atoms are asymmetric, which theoretically would allow cholesterol to exist in any one of 256 stereoisomeric forms. A solution of cholesterol would contain an equilibrium mixture of these different stereoisomeric forms. Illustrated in Figure 1 is the absolute configuration and conformation of cholesterol.



(Kice and Marvell, 1974)

Figure 1. Absolute configuration and conformation of cholesterol

Cholesterol - functions, sources, synthesis, metabolism.

Cholesterol functions as an essential stabilizing constituent of cell membranes, plasma lipoproteins and myelin (Myant, 1981). It is also the precursor of bile acids and steroid hormones and is essential for the growth and viability of cells. In the presence of light, cholesterol is converted into Vitamin D_3, cholecalciferol (Stryer, 1981).

Cholesterol is available for absorption from the intestine from three sources: bile, a small amount of de novo synthesis from the intestinal wall and from the diet. Dietary sources of this steroid consist of meat (including organ meats), poultry, fish, milk and its products, eggs and shellfish.

The major site of cholesterol synthesis in mammals is the liver. The rate of cholesterol synthesis is highly responsive to diet and is mediated by a change in the activity of 3-hydroxy-3-methylglutaryl CoA reductase which catalyzes the formation of mevalonate which is the committed step in cholesterol biosynthesis. Dietary cholesterol suppresses the synthesis of the reductase in the liver thus decreasing the rate of cholesterol biosynthesis. (Appendix A contains a simplified biochemical pathway for cholesterol formation).

In animals, cholesterol is metabolized primarily by conversion into bile acids, steroid hormones and by esterification with long-chain fatty acids. The major pathways of metabolism are qualitatively similar in avian and mammalian species. The differences are quantitative in the relative activities of the pathways and individual enzymes (Myant, 1981; Pearce, 1977; Stryer, 1981).

Cholesterol and steroid production.

The relationship between serum concentration of total cholesterol and ovarian steroid hormones was studied by Talavera et al. (1985). The experimental period of this study was through one complete estrous cycle in cattle during which total cholesterol, progesterone, testosterone and estradiol-17β were measured. They observed a decline in total serum cholesterol during the luteal phase of estrous which might result from the uptake and utilization of cholesterol by the luteal tissue. It was also thought that the luteal phase concentration of progesterone or some other component may suppress lipoprotein synthesis and/or the release of lipoprotein receptors. By alternating a control diet with a diet containing cholesterol through an estrous cycle and continuing to observe the decline in total serum cholesterol these researchers concluded that there was a modulation of lipoprotein synthesis and/or the release of factors associated with ovarian cyclicity. They also concluded that more than one pool of cholesterol seemed to exist in the bovine corpus luteum.

Using cholesterol-^{3}H in ovulating women, Bolte' et al. (1974) calculated the percentage of steroid derived from cholesterol. These researchers analyzed the metabolites (steroids), derived from the precursor (cholesterol), which were metabolized and excreted by 24 or 48 hours. Plasma cholesterol has a prolonged circulating half-life, whereas the metabolites have a short one. Using their data to calculate the percentage of steroid derived from plasma cholesterol, they concluded that plasma cholesterol was the major ultimate precursor of steroids and that other pathway(s), not in equilibrium

with plasma cholesterol, also contributed to steroid synthesis.

Cholesterol - levels.

Cholesterol levels vary from species to species. Table 1 illustrates this variance among several species.

Table 1. Blood cholesterol levels

SPECIES	MEAN CHOLESTEROL(mg/100ml)
Rabbit	40
Sheep	70
Mouse	97
Rat	50
Hog	110
Cat	95
Cockerel	100
Hen	116-152 (nonlaying) 208-285 (laying)
Human*	184
Rhesus monkey*	123
Dog*	138
Horse*	103
Goat*	68
Cow*	122
Guinea pig*	52
Trout*	245

*converted from mmol/l to mg/100ml

(Cook, 1958 and Terpstra et al., 1982)

The liver of a newly-hatched chick is rich in cholesterol, containing up to 1 gram of total cholesterol per 100 grams of fresh tissue. Most of the cholesterol present is esterified. As the bird matures, the liver cholesterol concentration decreases to values similar to those observed in the mammalian liver (Myant, 1981). Cholesterol levels in the hen, especially the laying hen, vary individually due to fat metabolism and ovulation periods, therefore,

conclusions regarding plasma cholesterol should be obtained from large replicated groups of birds (Johnson et al., 1959).

Non-genetic factors that influence blood cholesterol levels.

There are several non-genetic factors that exert an influence on blood cholesterol levels. Fasting can cause an increase in cholesterol levels (Hardy et al., 1962). As demonstrated in Table 2, the cholesterol level in the serum of chicks steadily increases with the duration of fasting.

Table 2. Effect of varied fasting time on serum cholesterol in chicks of high line (Four-week-old chicks at start of treatment. Number of birds per value ranged from 13 to 16. Mean serum cholesterol values in mg/100ml).

Sex	Duration of Fast, hr.					
	0	6	14	24	48	72
Male	204±8	218±12	202±11	257±12	269±10	298±11
Female	169±6	191±8	207±6	199±7	249±9	268±11

(Hardy et al., 1962)

Feeding diets containing either saturated or polyunsaturated fats leads to an increase of cholesterol synthesis approximately 12 hours after it is fed (Myant, 1981).

When pure cholesterol is added to the diet, an increase in the net absorption of cholesterol, resulting in a decrease in the rate of synthesis in the whole body was observed (Myant, 1981).

Confining layers in cages may tend to increase cholesterol in the blood plasma. Using isonitrogenous, isocaloric diets with varied levels of tallow and cerelose, Johnson et al. (1959) showed that

laying chickens can tolerate relatively large quantities of fat in their diet without having an appreciable increase in the cholesterol content of their blood plasma. A frequency distribution comparison between caged layers and layers on range, led these researchers to tentatively conclude that the higher blood cholesterol levels of the caged layers were the result of the birds' being kept in cages or the result of the combined effect of the birds' being kept in cages and receiving diets containing tallow.

Light intensity influences blood cholesterol level in birds. Polonis (1982b) conducted a series of experiments, involving all seasons of the year, with natural illumination of a medium intensity, 82.8 lux, versus artificial illumination, 47.7 lux, using a light cycle of 3 hours of light alternately with 1 hour of dark. He found a significantly higher cholesterol level in the plasma with artificial illumination at 5 weeks of age (82.4 mg% compared to 74.4 mg%) and a significantly lower cholesterol level in the plasma at 8 weeks of age, also with artificial illumination (92.4 mg% compared to 101.5 mg%).

Exogenous adrenocorticotropin hormone (ACTH) through its stimulatory effect on adrenal corticosteroid synthesis and release, has been shown to significantly increase cholesterol levels in the plasma of fowl (Siegel et al., 1984).

In wild birds, the end of the breeding season may also influence blood cholesterol levels. The seminiferous tubules have been observed to undergo a change involving a massive steatogenesis with the profuse appearance of cholesterol possibly due to the cessation of the synthesis of androgens (Lake and Furr, 1971).

Age also significantly influences blood cholesterol levels. Work by Estep et al. (1969), and further supported by Polonis (1982a), determined that cholesterol significantly increases with age.

Hardy et al. (1962) developed a high and a low serum cholesterol line in chickens for studies to determine the underlying mechanism for differences in serum cholesterol in these lines. Both lines reacted similarly to fasting, temperature stress and to most of the hormones administered, so it appeared improbable that a response to diet or a difference in intestinal absorption was responsible for the line differences. The results of most of their other experiments were negative, with respect to providing information on the underlying mechanism for the line differences.

Yolk cholesterol.

The relationship between yolk and serum cholesterol is as yet unknown. Some selection studies on yolk and serum cholesterol have shown the changes in the two to be parallel while other studies have shown no relationship. The majority of egg yolk cholesterol originates from serum cholesterol and there is some indication that the ovarian follicle may produce some cholesterol in vitro (Washburn, 1975).

The high and low plasma cholesterol lines that Hollands et al. (1980) developed in White Leghorns were evaluated for egg yolk cholesterol. The low plasma cholesterol line had substantially lower egg yolk cholesterol than the unselected line; however, the high plasma cholesterol line did not have a substantially higher egg yolk

cholesterol than the unselected line, in fact it had a slightly lower egg yolk cholesterol than the unselected line.

In a divergent selection program for high and low yolk cholesterol, Marks and Washburn (1977) reported that selection could effectively change the cholesterol concentration only in an upward direction. Ansah et al. (1985) selected for low yolk cholesterol but the changes were relatively small. In 1977, Ali reported a significant negative genetic correlation between the level of egg yolk cholesterol and blood serum cholesterol level. The phenotypic correlation between these traits was small and would be considered zero.

Genetic selection for blood cholesterol levels in animals.

Genetic selection for high and low serum cholesterol has been successful in mice (Dunnington et al., 1981a,b). The most pronounced effect of serum cholesterol in mice was on female body weight. There was a significant increase in 12-, 42-, and 56-day weight in females with high serum cholesterol and a significant decrease in 56-day weight in females with low serum cholesterol. There was no change in the percent of females littering, the average littering interval or the number of live pups at 5 days. Realized heritabilities were 0.41±0.05 and 0.44±0.05 for males and females respectively in the high cholesterol line and 0.37±0.05 and 0.37±0.06 for males and females respectively in the low cholesterol line.

Selection for high and low serum cholesterol level has also been successful in swine. Rothschild and Chapman (1976) reported a

heritability of approximately 0.25 in swine. After two generations of selection there were significant differences between the high and the low cholesterol lines. Body weight and dam had a significant effect on the serum cholesterol level, whereas age of pig, sex, farrow, sire or litter size had no significant effects.

In studies of serum cholesterol levels in dairy cattle, Arave et al. (1975) reported a heritability of 0.50 for first lactation cows and 0.19 for heifers from 2 to 22 months of age. These researchers found correlations between body weight and cholesterol and also between age and cholesterol.

Genetic selection for blood cholesterol levels in White Leghorns.

Genetic selection for blood cholesterol levels in White Leghorns has been performed by a number of researchers. Following three generations of selection, Cherms et al. (1960) developed high and low serum cholesterol lines of White Leghorns that differed markedly in mean serum cholesterol levels. The development of these lines was by selection of extreme individuals within extreme families. The purpose of creating these lines was to determine if age significantly affected serum cholesterol levels. These researchers found that there was no significant variation in cholesterol levels between 1 and 10 weeks of age. Heritability estimates obtained for these lines were 0.19 from the sires; 0.41 from the dams and a combined sire x dam heritability of 0.30.

Wilcox et al. (1963) studied the relationship between serum cholesterol level and productive performance. Blood cholesterol was

measured at 6 weeks of age and then again at 8 months of age. It was found that there was a statistically significant positive correlation ($p \leq 0.05$) between the cholesterol level of the young pullet and the adult cholesterol level. Positive correlations between cholesterol level and body weight at 8 weeks of age, age at first egg, egg production and albumen quality were also observed. A negative correlation was found to exist between cholesterol level and adult body weight. Heritability estimates of serum cholesterol obtained by these researchers from the birds at 6 weeks of age were 0.34 from the sires; 0.17 from the dams and a combined sire x dam heritability of 0.25.

Wilcox and Shaffner (1963), studied the performance of lines selected for high and low 6 week serum cholesterol levels. Their results, as presented in Table 3, led these researchers to conclude that selection for high or low serum cholesterol in the young chicken results in little, if any, improvement of productive traits. They found no differences between the high and low cholesterol lines with respect to adult body weight, mortality, fertility, hatchability, egg weight, or specific gravity. Consistent differences were found between these lines for body weight at 6 weeks of age and Haugh units.

Table 3. Performance of lines selected for high and low cholesterol and a random bred control

Line	Year Hatched	Serum Cholesterol Level, 6 wk. (mg/100ml)	Body Wt. 6 wk. (g)	Body Wt. 1 yr. (lb)	Egg production hen day basis 1st yr. (eggs/yr)	Mortality 1st laying yr.(%)	Fertility (%)	Hatchability (%)	Egg Wt. (g)	Specific Gravity	Haugh Units
Low	1958	120	469	4.6	245	16	90	89	59	1.077	70
	1959	127	391	4.4	253	7	74	91	58	1.077	67
	1960	135	458	4.2	202	13	87	74	57	1.079	71
	1961	99	451	4.6	211	27	78	79	-	-	-
	Avg.	120	442	4.5	228	16	82	83	58	1.078	69
Random Bred	1958	134	483	4.5	221	14	-	-	61	1.078	73
	1959	151	370	4.4	242	13	-	-	61	1.079	72
	1960	146	496	4.5	250	15	-	-	59	1.080	73
	1961	113	435	4.6	241	14	-	-	-	-	-
	Avg.	136	446	4.5	239	14	-	-	60	1.079	73
High	1958	145	449	4.4	234	14	85	90	56	1.079	74
	1959	160	381	4.5	255	20	83	87	57	1.077	72
	1960	159	429	4.4	246	35	87	85	57	1.078	74
	1961	129	438	4.8	246	19	76	81	-	-	-
	Avg.	148	424	4.5	245	22	83	86	57	1.078	73

(From Wilcox and Shaffner, 1963)

Estep et al. (1969) undertook a study to determine the effect of age on serum cholesterol levels. In their study, only males were used to avoid any variable influence from egg production. Blood samples were first collected at 6 weeks of age and then at 2 week intervals until the birds reached 20 weeks of age. From 6 weeks to 12 weeks of age, the mean serum cholesterol levels decreased by 6.4mg% per week. From 12 weeks to 20 weeks of age, the mean serum cholesterol levels increased by 5.6mg% per week, which may have been due to metabolic changes associated with the onset of sexual maturity. These results, contradictory to the results of Cherms et al. (1960), indicated that age was highly significant. These researchers further concluded that the correlation between 6 week and 20 week serum cholesterol values was significant enough to select birds at 6 weeks of age for 20 week high and low serum cholesterol values.

Hollands et al. (1980) studied the response to five generations of selection for blood cholesterol levels. Birds were selected by their cholesterol level and high and low lines for blood cholesterol level were successfully developed. At 9 to 10 weeks of age, males were found to have a significantly higher cholesterol level than females of the same age, indicating a sexual dimorphism. Also noted, was a small, but consistent, negative correlation between cholesterol level and egg production which contrasts with the findings of Wilcox et al. (1963) previously discussed. Due to severe losses from Marek's disease during the term of this experiment, the relationship between cholesterol level and mortality was examined. It was concluded that lower cholesterol levels conferred a small but "real"

biological advantage for a birds livability.

Japanese Quail as a pilot animal for genetic research.

Bantam chickens, because of their small size, have been advocated for use as a pilot animal for poultry research but they lack several important characters of a successful pilot animal. They are characterized by a slower growth rate and a lower rate of egg production than the usual chicken varieties (Wilson et al., 1961). Japanese Quail (Coturnix coturnix japonica) on the other hand, might alleviate limits of budget, time and space. They are hardy, resistant to most common avian diseases and easy to handle. The Japanese Quail is characterized by a high rate of egg production and a rapid generation turnover, producing three to four generations per year. They are relatively inexpensive, simple to feed and eight to ten quail can be housed in the same amount of space as one large chicken. They have numerous physiological characteristics similar to chickens and turkeys and can be housed in modified chicken or turkey facilities (Ernst; Wilson et al., 1961; Woodard et al., 1965). Table 4 compares various characteristics of Coturnix with Leghorn chickens and Broad Breasted Bronze turkeys.

By modifying a regular chicken egg incubator tray with wire, an incubator with the capacity to hold 2500 chicken eggs can hold 4200 Japanese Quail eggs. Embryonic mortality of the Japanese Quail closely resembles chickens and especially turkeys and occurs mostly during two periods - the first two days of incubation and just prior to hatching. Upon transferring the eggs to hatching baskets it is

recommended that cheese cloth be placed on the bottom to prevent the occurance of spraddled legs.

Chicken brooder batteries can be modified to accomodate Japanese Quail during the brooding stage. A smaller wire mesh than is used in chicken brooders needs to be placed on the floor and pebbles, marbles or wire needs to be placed in the waterers to prevent accidental drowning. Sex determination by breast plumage color (male - cinnamon; female - speckled - white with black spots) is possible as early as two weeks of age but a greater accuracy is achieved at three weeks of age.

Table 4. Comparison for various characteristics of Coturnix with Leghorn chickens and Broad Breasted Bronze turkeys

CHARACTERISTIC	COTURNIX	CHICKEN	TURKEY
Body weight at hatch, gm.	6.2	40	59
Body weight at sexual maturity, kg.			
Males	0.105	1.50	10.0
Females	0.115	1.45	6.8
Full adult body weight, kg.			
Males	0.110	2.30	15.4
Females	0.130	1.70	8.4
Av. age at first egg, weeks	6	23	33
Egg weight during maximum production, gm.	9.5	55	85
Egg weight as percentage of adult female body weight	7.0	3.2	1.0
Pounds of feed per pound of eggs at maximum lay	3.0	3.0	5.5
Maximum rate of lay (percentage)	70-90	70-80	55-65

(Wilson et al., 1961)

The disadvantages of using Japanese Quail are the difficulty of wing-banding day old chicks for identification, drowning in the waterers, feed wastage, cannabilism and inbreeding depression.

Japanese Quail are more sensitive to the effects of inbreeding than are other fowl. The adverse effects of inbreeding are the most pronounced for fertility of eggs and hatchability of fertile eggs. Hatchability decreases approximately one percent for each percent increase of the inbreeding coefficient (Woodard et al., 1965).

MacNeil et al. (1984) conducted a selection experiment for seventeen generations to study inbreeding and fitness in Japanese Quail. They compared three different mating systems - 1. a selected population mated in a cyclic manner with alternating generations of inbreeding and outbreeding; 2. a selected population mated at random and 3. a randomly mated, randomly selected control population. They observed a loss of fitness with continued inbreeding and a trial, to study the effects of previous mating systems on the response to continuous inbreeding, initiated at generation twelve was terminated after two generations because no families had sufficient members to continue.

Sittmann et al. (1966) also studied inbreeding depression in Japanese Quail. They bred for high levels of homozygosity at a relatively slow rate to permit the elimination of deleterious genes and gene combinations by natural selection. There was no selection for any traits. Their findings were as follows: The total number of eggs decreased by 1.5 eggs for each 10% of inbreeding. The decrease in the hatch of fertile eggs was approximately 46% when the inbreeding

coefficient, F, was equal to 0.5. Maternal inbreeding caused a decrease in hatchability of approximately 3% for each 10% F and also an increase of early mortality. There was also an increase in early mortality when the embryo was inbred. The hatchability of inbred embryos decreased approximately 7% for every 10% increment of inbreeding. Mortality up to the fifth week of age increased approximately 2% and 4% for each additional 10% inbreeding of the dam and offspring, respectively. In the inbred female, sexual maturity was delayed by slightly more than one day for each 10% of inbreeding. Body weight, at 6 weeks of age, was depressed by 2 grams in males and 4 grams in females with 10% inbreeding of the progeny. The depression of 6 week body weight is more apparent in the female because of the greater weight of the reproductive organs of the female.

MATERIALS AND METHODS

The parent generation of Japanese Quail for this study was derived from the randomly mated population maintained at Oregon State University Department of Poultry Science. Eggs were collected for one week and incubated resulting in 194 chicks (106 females and 88 males) from which to choose a parental group of birds numbering 100 (50 females and 50 males). At the time of hatch, the chicks were wing banded with self-piercing wing bands for identification.

The chicks were brooded in a standard Mother Hen Brooder from Georgia Quail Farms. At 4 weeks of age the birds were transferred to colony pens until breeder selection, at which time the birds were transferred to individual battery breeding pens. The breeding pens consisted of 5 rows of 6 pens (25.5 cm x 61 cm x 21.5 cm each pen) and each generation was completely random as to the placement of breeding pairs in the breeding pens. The brooder, colony pens and breeding pens were all located within the same positively ventilated room.

Lighting was provided continuously and feed and water were provided ad libitum throughout the study. The chicks were fed a Japanese Quail Starter (O.S.U. ration #1876) containing 28.7% crude protein and 2812 Kcal/kg. metabolizable energy (ME) from hatching to three weeks of age. At three weeks of age, the ration was changed to a broiler starter (O.S.U. ration #1476) containing 20.3% crude protein and 2911 Kcal/kg. ME. From six weeks of age, the birds were fed a Japanese Quail Breeder (O.S.U. ration #1771) containing 24.1% crude protein and 2845 Kcal/kg. ME (The compositions and calculated analyses of the above feeds are contained in Appendix B).

Blood Analysis.

At four weeks of age approximately 0.5cc of blood was drawn by cutaneous ulnar vein puncture and collected into sterile glass tubes containing 75 IU sodium heparin. The birds were offered no special treatment in terms of fasting, water denial, etc. Blood was drawn at approximately the same time of day in each generation.

Blood samples were centrifuged at 1500 x g for 15 minutes, the plasma removed and stored at -25°C in rubber stoppered glass tubes. Analysis of blood cholesterol was performed using a Cholesterol Test Kit from Boehringer Mannheim Diagnostics (Indianapolis, IN). Prior to analysis, the frozen plasma samples were allowed to thaw at room temperature. Differences between frozen-thawed and fresh plasma cholesterol levels were determined not to be significant and freezing the plasma allowed for uniform testing from generation to generation. Procedural modifications, of the cholesterol assay, consisted of using a 10 lambdaliter plasma sample and 1 ml. of working reagent containing the following:

CHOLESTEROL REAGENT
Reactive Ingredients

100	mM	Tris buffer, pH 7.7
50	mM	Magnesium aspartate
1	mM	4-Aminophenazone
6	mM	Phenol
4	mM	3,4-Dichlorophenol
0.3%		Hydroxypolyethoxy-n-alkanes
>400	U/L*	Cholesterol esterase (25°C); EC 3.1.1.13, microorganism
>250	U/L	Cholesterol oxidase (25°C); EC 1.1.3.6, Nocardia erythropolis
>200	U/L	Peroxidase (25°C); EC 1.11.1.7, horseradish

Nonreactive stabilizers have been added
*U/L = units per liter

The plasma and reagent were allowed to react in a 37°C water bath for 10 minutes to produce a chromogen and spectrophotometrically analyzed for cholesterol concentration at a wavelength of 505 lambda. The spectrophotometer was programmed, using cholesterol reference solutions from the Boehringer Mannheim Company, to prepare a calibration curve, ranging from 50 to 400 mg/dL, to allow for a direct measurement of cholesterol concentration in the samples.

The principles of the Boehringer Mannheim Diagnostics Cholesterol Test Kit are as follows: Cholesterol esters in the plasma, plus water are converted to cholesterol and fatty acids through the action of a cholesterol esterase. The cholesterol combines with a molecule of oxygen and through the action of cholesterol oxidase is converted to cholest-4-en-3-one and hydrogen peroxide. Two molecules of hydrogen peroxide join with 4-aminophenazone and through the action of a peroxidase is converted to a p-quinone imine dye, the intensity of which is proportional to the concentration of cholesterol in the sample. The use of cholesterol esterase and cholesterol oxidase in combination with a color reaction increases the specificity and sensitivity of this procedure (Kovar and El-Yazbi, 1983). A comparison by Deacon and Dawson (1979) between chemical hydrolysis and enzymic hydrolysis for a total cholesterol assay showed that chemical hydrolysis methods yielded results that were about 10% lower than those obtained by enzymic hydrolysis. These results were because of the incomplete removal of interfering thiols generated during the saponification of serum. Furthermore, enzymic assay is simpler and lends itself to automation.

Selection.

A preliminary study was conducted to determine the optimum time to select the Japanese Quail based on their blood cholesterol levels. Blood was drawn at hatching, 1, 2, 3, 4, 5, 6, 8, 10, 14 and 18 weeks of age. Equal numbers of males and females were used at each age. Because of their small size, the quail were sacrificed for the blood samples collected at hatching, 1 and 2 weeks of age. Different quail were used for each of the blood samples drawn by cutaneous ulnar vein puncture at 3, 4 and 5 weeks of age. All blood samples collected from weeks 6 to 18 were from the same group of quail and were obtained by cutaneous ulnar vein puncture.

For this study, five lines of birds were created with ten pair matings per line. Birds were selected based upon individual blood cholesterol levels determined at 4 weeks of age. A control line was developed first by random selection from the parent generation. Birds with the highest blood cholesterol levels were chosen for the simultaneous creation of the duplicate high lines and birds with the lowest blood cholesterol levels were chosen for the simultaneous creation of the duplicate low lines. Birds were paired according to blood cholesterol level, avoiding sib matings. Beak rings were employed to decrease the incidence of cannibalism and records were kept so that each bird was fully pedigreed. In addition to the selection of breeding pairs extra birds were chosen and held in reserve in the event that the breeding pair did not produce eggs or one of the pair expired.

Approximately two weeks after creating breeding pairs, eggs were

collected, marked by pen number and stored in a cooler at 13°C. After a week of saving the eggs, they were incubated in a single stage 252 Jamesway Incubator. In the event that something happened to these eggs during the incubation process a second set of eggs was saved and incubated. Eggs that did not hatch were broken out and examined macroscopically in an attempt to determine the cause and also to determine true fertility. Any embryonic disorders were studied with additional weekly egg settings from the breeding pair, to determine if the disorders might have been associated with the cholesterol line.

Production records were kept as follows: egg production - for a six week period beginning when the birds were 7 weeks of age, hatchability of fertile eggs and of all eggs, percent fertility and percent mortality (from hatch to 6 weeks of age). The final generation of selected birds (generation 4) was also weighed at 9 weeks of age.

Statistical Methods.

Measurements of % Fertility, % Hatch of Fertile Eggs (%HFE), % Hatch of All Eggs (%HAE) and % Production were calculated on a per hen basis and % Mortality was calculated on a per line basis as follows:

$$\%\ \text{Fertility} = \frac{\text{total number of eggs set} - \text{number of infertile eggs}}{\text{total number of eggs set}} \times$$

$$\%\ \text{HFE} = \frac{\text{number of hatched chicks}}{\text{number of fertile eggs}} \times$$

$$\%\text{HAE} = \frac{\text{number of hatched chicks}}{\text{total number of eggs set}} \times$$

% Production = total number of eggs produced ÷
total number of days from first egg after pairing until end of collection

% Mortality = total number of birds dead ÷
total number of hatched chicks

(calculated up to approximately 6 weeks of age)

Heritability (h^2) estimates, based on intraclass correlations and the standard error of these estimates (S.E. h^2) were calculated according to Becker (1984). The statistical model used was:

$$Y_{ik} = \mu + \alpha_i + e_{ik}$$

where μ is the common mean
α_i is the effect of the i-th mating
e_{ik} is the uncontrolled environmental and genetic deviations attributable to individuals within single pair matings

All effects are assumed to be random, normal and independent with expectations equal to zero.

The Analysis of Variance Table is as follows:

Source of Variation	d.f.	SS	MS	EMS
Between matings	s-1	SS_s	MS_s	$\sigma_w^2 + k_1 \sigma_s^2$
Between progeny, within matings	n.-s	SS_w	MS_w	σ_w^2

s = number of matings
k_1 = number of individuals within the i-th mating in the expected mean squares
n. = total number of individuals

From the ANOVA table, heritability was calculated as follows:

$$h^2 = \frac{2\,\hat{\sigma}_s^2}{\hat{\sigma}_s^2 + \hat{\sigma}_w^2}$$

where: $\hat{\sigma}_s^2 = \frac{MS_s - MS_w}{k_1}$

MS_s = mean square between matings
MS_w^s = mean square between progeny, within matings

k_1 = number of individuals per mating (unequal)

$$= \frac{1}{s-1}\left(n_\bullet - \frac{\Sigma n_i^2}{n_\bullet}\right)$$

n_i = number of individuals in i-th mating

$$\hat{\sigma}_w^2 = MS_w$$

$$S.E.(h^2) = 2\sqrt{\frac{2(n_\bullet-1)(1-t)^2[1+(k_1-1)t]^2}{k_1^2(n_\bullet-s)(s-1)}}$$

t = intraclass correlation

$$= \frac{\hat{\sigma}_s^2}{\hat{\sigma}_s^2 + \hat{\sigma}_w^2}$$

A regression of the cumulative response on the cumulative selection differential was performed to determine realized heritability over the generations according to the method described by Falconer (1981).

Correlation coefficients (Pearson's r) were calculated to determine if significant relationships existed between cholesterol level and various production parameters (%Fert, %HFE, %HAE, %Prod,

%Mortality and Weight in grams).

The percent inbreeding of the final generation (generation 4) was calculated according to Wrights Inbreeding Coefficient (Falconer, 1981):

$$F = \Sigma(1/2)^{n} (1+F_{A})$$

Where: n = number of birds involved in the pedigree "path"

F_{A} = the % inbreeding of the common ancestor

RESULTS AND DISCUSSION

The results of the preliminary study conducted to determine the optimum time to select the Japanese Quail based on their blood cholesterol levels is represented in Figure 2. It was determined that 4 weeks of age would be the optimum time for cholesterol evaluation and selection because at this age the birds are old enough to be accurately sexed but young enough so that the variable influences of sexual maturity are not too evident in blood cholesterol values.

The numbers of birds available for selection, after chick mortality (0-6 weeks), were as follows:

	Generation							
	1		2		3		4	
Line	M	F	M	F	M	F	M	F
Control	29	15	16	26	12	9	19	14
Low #1	21	9	14	13	18	14	19	18
Low #2	10	18	16	15	16	18	22	16
High #1	14	14	19	23	12	17	13	17
High #2	19	19	19	26	18	19	12	14

After four generations of genetic selection for high and low blood cholesterol, lines of birds were created that differed significantly in their respective blood cholesterol levels. The quantity of cholesterol present in the plasma, over the generations, of all birds and of selected birds is presented in Tables 5 - 10. Figures 3 and 4 represent these quantities graphically.

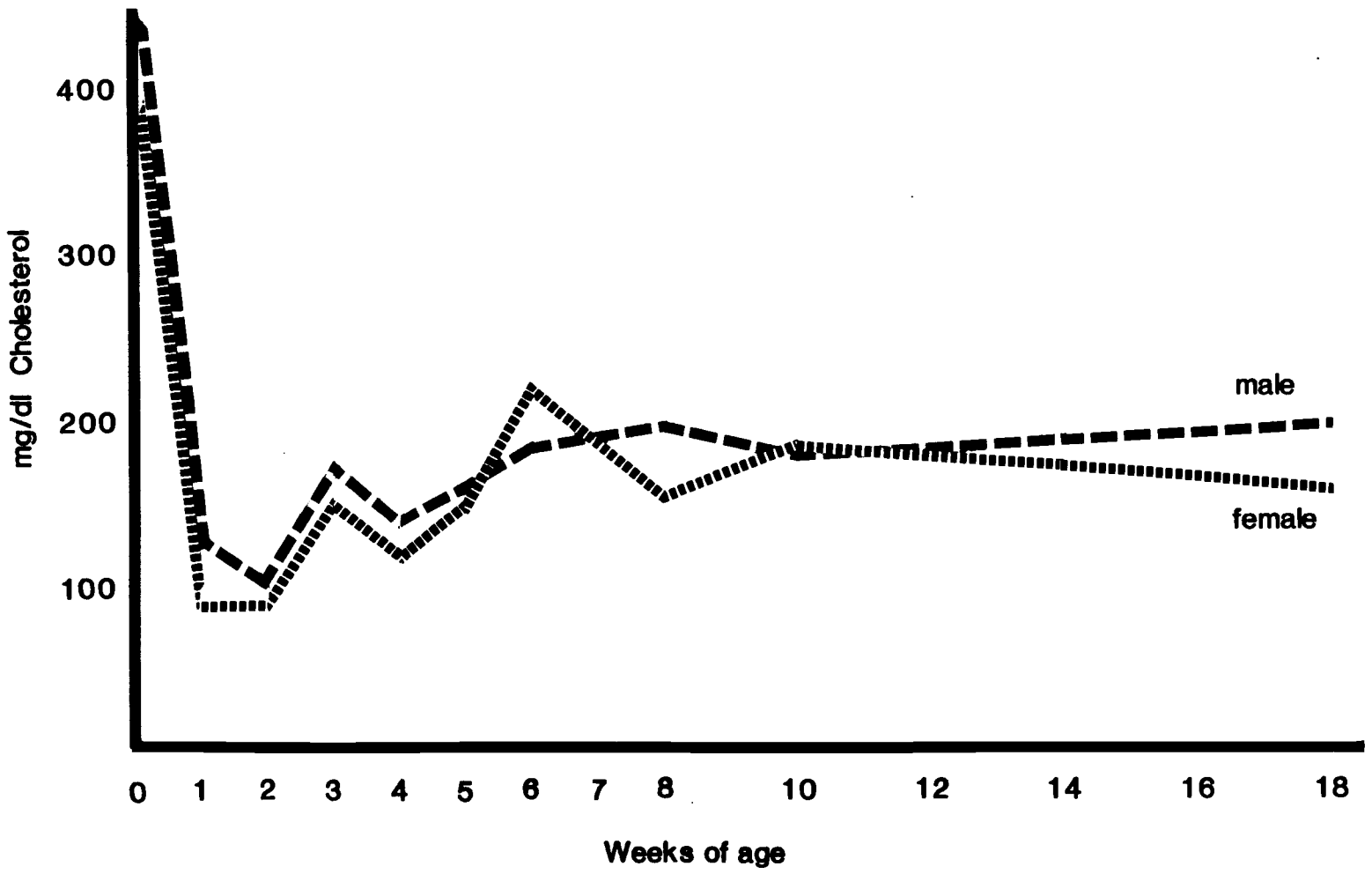


Figure 2. Preliminary age/sex study of cholesterol levels in Japanese Quail

Table 5. Mean cholesterol (mg/dL) levels for unselected birds (4 weeks of age) - male and female.

	Generation				
Line	Parents	F1	F2	F3	F4
C	155.6±15.2	150.5±25.7	127.9±32.2	101.3±18.1	123.9±20.2
L1	120.2±17.0	133.3±31.2	113.3±21.3	100.0±16.1	117.6±31.5
L2	125.9±16.6	128.0±24.4	124.3±23.1	100.0±19.9	133.1±28.3
H1	196.8±38.5	146.5±21.1	147.8±30.5	114.8±18.3	144.3±34.2
H2	198.4±28.7	158.9±31.2	163.1±37.8	133.8±19.5	170.3±55.5

Table 6. Mean cholesterol (mg/dL) levels for birds selected as breeders (4 weeks of age) - male and female

	Generation				
Line	Parents	F1	F2	F3	F4
C	155.4±16.1	146.8±20.9	132.7±29.6	97.2±16.4	120.6±19.1
L1	120.2±17.0	111.8±14.5	108.1±16.1	91.1±11.5	102.0±15.2
L2	125.9±16.6	128.1±28.6	118.5±12.7	93.3±10.8	112.7±16.0
H1	196.8±38.5	155.7±17.4	169.8±26.0	123.7±16.3	159.6±30.8
H2	198.4±28.7	178.7±26.3	194.9±29.1	140.6±16.2	172.3±31.2

(values are mean ± standard error of the mean)

Table 7. Mean cholesterol (mg/dL) levels for unselected birds (4 weeks of age) - male

	Generation				
Line	Parents	F1	F2	F3	F4
C	165.7± 8.4	156.8±25.7	136.6±28.5	106.7±17.0	130.2±17.4
L1	131.3±11.1	143.7±29.5	116.5±20.9	104.6±13.7	119.7±10.0
L2	138.2± 9.3	146.7±26.4	135.3±20.6	109.2±22.5	138.0±22.6
H1	210.6±38.9	153.2±16.7	157.8±29.9	129.9±15.5	158.9±41.1
H2	210.7±29.1	170.3±34.7	156.5±29.0	144.2±14.7	170.1±36.1

Table 8. Mean cholesterol (mg/dL) levels for birds selected as breeders (4 weeks of age) - male

	Generation				
Line	Parents	F1	F2	F3	F4
C	167.7± 7.9	155.4±10.1	156.6±19.2	106.5±15.4	126.6±14.7
L1	131.3±11.1	118.7± 7.0	107.4±12.2	93.7± 7.8	112.2± 6.1
L2	138.2± 9.3	148.2±27.5	124.5±11.9	98.7± 9.6	119.0±13.5
H1	210.6±38.9	161.4±15.0	178.0±32.1	134.3±15.3	170.3±40.2
H2	210.7±29.1	194.3±25.2	179.2±15.5	152.0± 8.3	179.9±31.0

(values are mean ± standard error of the mean)

Table 9. Mean cholesterol (mg/dL) levels for unselected birds (4 weeks of age) - female

	Generation				
Line	Parents	F1	F2	F3	F4
C	143.1±12.1	138.3±21.7	122.6±33.7	92.4±17.5	115.3±21.1
L1	109.1±14.6	108.8±19.8	109.8±22.0	94.0±17.4	115.5±44.6
L2	113.7±12.7	117.6±16.0	112.5±20.0	91.7±13.0	126.4±34.2
H1	183.0±35.4	139.8±23.4	139.6±29.2	104.3±12.5	133.2±23.4
H2	186.1±23.5	147.6±22.9	168.0±43.1	124.0±18.6	170.4±69.5

Table 10. Mean cholesterol (mg/dL) levels for birds selected as breeders (4 weeks of age) - female

	Generation				
Line	Parents	F1	F2	F3	F4
C	143.1±12.1	138.2±25.7	108.8±14.2	87.9±12.0	114.6±21.7
L1	109.1±14.6	105.0±17.1	108.8±20.0	88.6±14.4	91.8±14.9
L2	113.7±12.7	108.0± 8.4	112.4±11.0	87.8± 9.3	106.5±16.4
H1	183.0±35.4	149.9±18.6	161.5±16.2	113.2± 8.8	148.8±11.6
H2	186.1±23.5	163.1±17.0	210.5±31.6	129.2±13.9	164.7±31.1

(values are mean ± standard error of the mean)

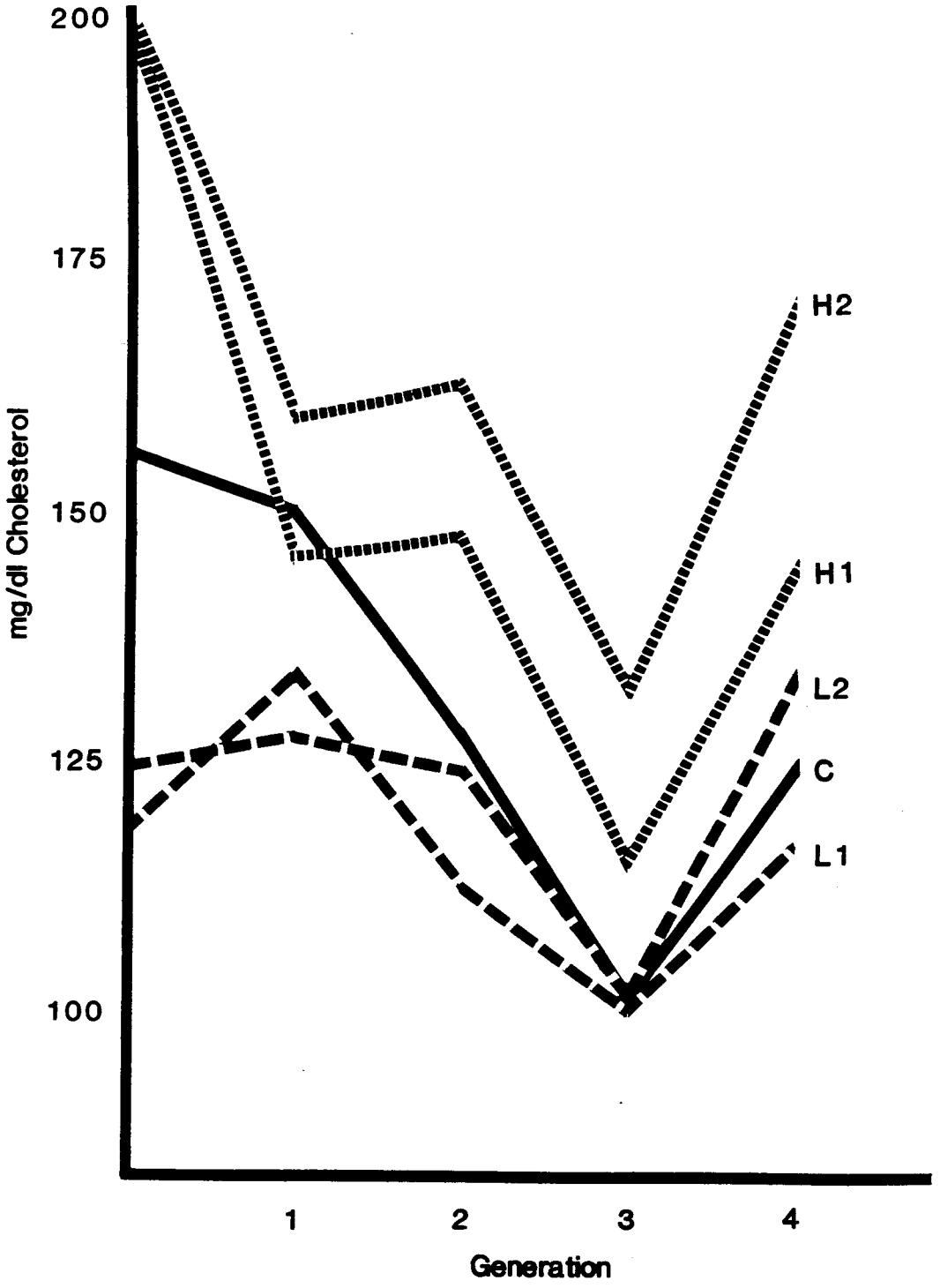


Figure 3. Mean cholesterol levels of unselected Japanese Quail (4 weeks of age)

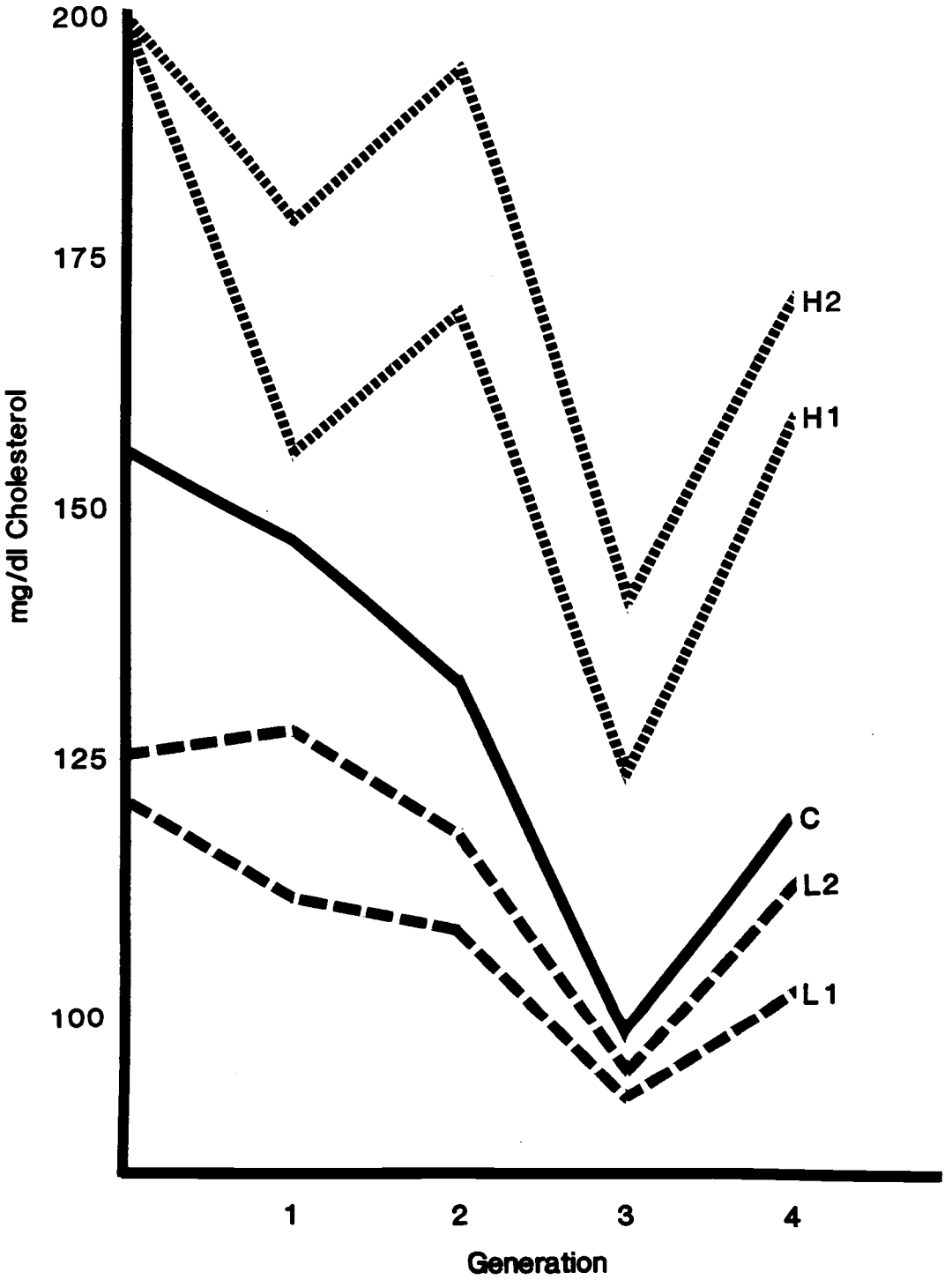


Figure 4. Mean cholesterol levels of selected Japanese Quail (4 weeks of age)

The cholesterol level (mg/dL) of the selected birds as a deviation from the control is represented in Figure 5. Presentation of the data in this manner allows one to view the progress of a selection program that has been corrected for environmental effects (Richardson et al., 1968). The high blood cholesterol lines of birds responded in a generally upward direction. The low blood cholesterol lines of birds also responded in an upward direction but with less fluctuation than the high lines. It wasn't until the last generation (generation 4) that a decrease in blood cholesterol levels was seen in the low blood cholesterol line. There were no significant differences between the replicates of the high and the low blood cholesterol lines so data from the replicated lines was pooled and there were found to be significant differences between the high and the low blood cholesterol lines ($p \leq 0.01$). Differences between the males and the females were only significant in the control line ($p \leq 0.05$).

Heritabilities (h^2) and the standard error of these estimates (S.E. h^2), as calculated for each line of generation 4 from Analysis of Variance components, are as follows: Low #1 = -0.25 ± 0.19; Low #2 = 0.11 ± 0.32; High #1 = 0.31 ± 0.41; High #2 = 0.45 ± 0.45. The large standard errors reflected in the heritability calculations may be due to small numbers of birds in each line which showed a large amount of variability.

Realized heritabilities as determined by regression analysis and estimated over the generations are as follows: Low #1 = 0.38; Low #2 = -0.22; High #1 = -0.22; High #2 = -0.10. The comparison of the regression lines calculated for each of the blood cholesterol lines of

birds showed both the high and the low lines to differ significantly from the control line ($p \leq 0.05$), which as a regression line would be considered to have a slope of 0. Realized heritability is presented here primarily as a descriptive "response to selection" estimation. Changes due to environmental trends, inbreeding and random drift tend to confound the response (Falconer, 1981). The standard errors have been omitted from these heritability estimates due to the fact that they are the standard errors of the regression line and not of the cholesterol line itself. The standard error of the regression line does not account for the variation arising from random drift - as changes due to random drift are cumulative.

A summary of production parameters, mortality and body weight are presented in Table 11 (This table contains information for the final generation only. For information regarding other generations see Appendix C). While the levels of blood cholesterol between the high and the low lines of birds were found to be significantly different, production parameters were found to be not significantly different between the high and the low blood cholesterol lines.

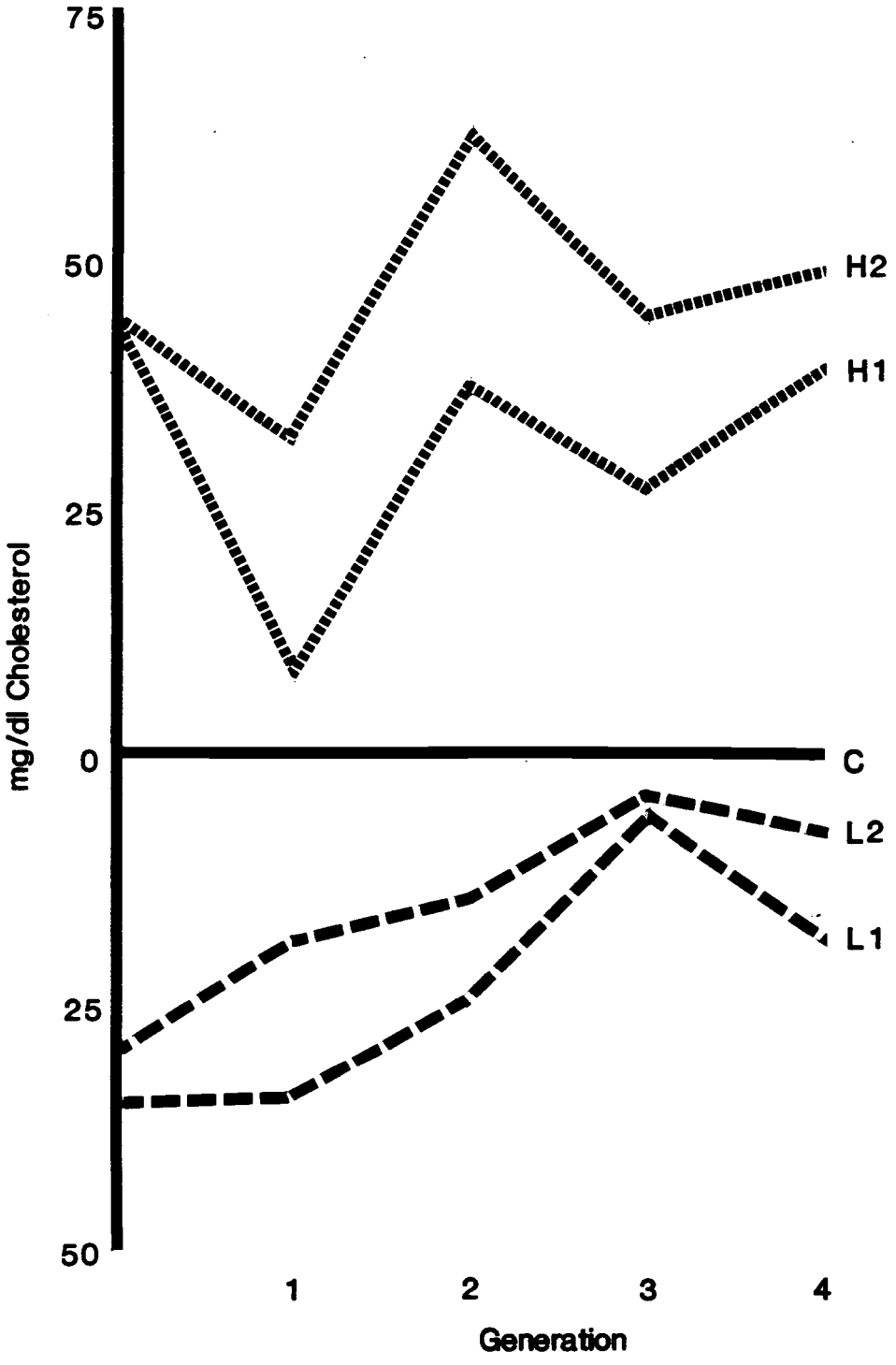


Figure 5. Mean cholesterol levels of selected Japanese Quail (4 weeks of age) as a deviation from the control

Table 11. Summary of production parameters, mortality and body weight for lines selected for blood cholesterol levels

Line	Avg. mg/dL Cholesterol[a]	%Prod[b]	%Fert	%HFE	%Mort[c]	Weight(g)[d]
C	120.6	82.5	94.6	80.5	17.5	108.0
L1	102.0	82.2	95.6	83.1	17.0	118.4
L2	112.7	80.9	85.3	67.1	11.6	116.8
H1	159.6	78.8	97.2	86.3	9.1	116.5
H2	172.3	86.4	82.6	71.4	22.9	115.4

a - blood sampled at 4 weeks of age
b - 6 week period beginning when birds were 7 weeks of age
c - from hatch to 6 weeks of age
d - 9 weeks of age

Correlation coefficients were calculated to determine if significant relationships existed between production parameters and blood cholesterol level. Using the combined calculated correlations it was determined that cholesterol level was negatively and significantly correlated with 9 week body weight ($p<0.05$).

Since Japanese Quail are very sensitive to inbreeding, the percentage of inbreeding was calculated using Wrights F. Due to the small number of quail in each line and the number of generations covered some inbreeding did occur in the later generations in retrospect to the earlier generations. The largest F value encountered in each line was F = 0.0938 (9.0%). By avoiding full-sib matings each generation, the retrospective amount of inbreeding was decreased with outbreeding.

Some of the embryonic disorders encountered during this experiment were: posterior duplications with cranial hernia, twisted beaks, missing limbs and shortened neck, legs and body. The settings of additional eggs to study any possible relationship between

cholesterol levels and these embryonic disorders produced all normal embryos which leads to the conclusion that the disorders may have been induced by the incubation process.

SUMMARY AND CONCLUSIONS

This experiment resulted in the creation of lines of Japanese Quail that differed significantly in their respective blood cholesterol levels beginning with the first generation and continuing for the duration of this experiment. Had this experiment been allowed to continue for several more generations a more dramatic divergence between the high and the low blood cholesterol lines may have become evident especially with regards to the high blood cholesterol lines of birds.

The low blood cholesterol lines of birds seem to have responded from the physiologically lower limit of cholesterol level required for growth and reproduction. Since cholesterol is necessary for the very "heart" of life itself - the growth and viability of cells - it seems only fitting that these lines of birds would respond to a "self-preservation" form of natural selection in addition to the artificial selection being applied. As a result, the low blood cholesterol lines of birds have responded with an upward trend in blood cholesterol level. Heritability estimates of the low lines were - Low #1 = -0.25 ± 0.19 and Low #2 = 0.11 ± 0.32.

The high blood cholesterol lines of birds responded in a generally upward direction. The physiological upper limit of blood cholesterol probably would require several more generations to achieve. Mortality associated with high levels of blood cholesterol was observed to occur at levels far exceeding the levels present in the high blood cholesterol lines of birds in this experiment (Personal observations during the pre-trial period). Heritability

estimates of the high lines were - High #1 = 0.31 ± 0.41 and High #2 = 0.45 ± 0.45.

The production parameters of egg production, fertility, hatch of fertile eggs and mortality and body weight did not differ significantly between the high and the low blood cholesterol lines. There was found to be a significant negative correlation between blood cholesterol level and 9 week body weight.

The results of this experiment cannot be interpreted by themselves. Due to the varying results of other experimentation in the genetic selection for blood cholesterol, these results must be interpreted as a part of the sum research conducted in this area.

The use of blood cholesterol levels in the avian species could perhaps best be as one of several traits used in the selection process. Since blood cholesterol levels have been shown, in some cases, to be positively correlated with certain aspects of production, one could select birds whose cholesterol levels range from an established intermediate to a medium-high blood cholesterol level. This trait, along with others, could then be used to improve production in a particular line of birds.

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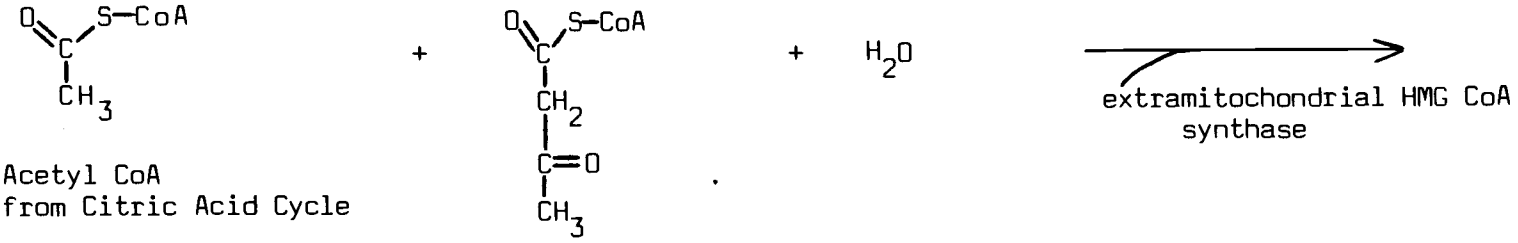
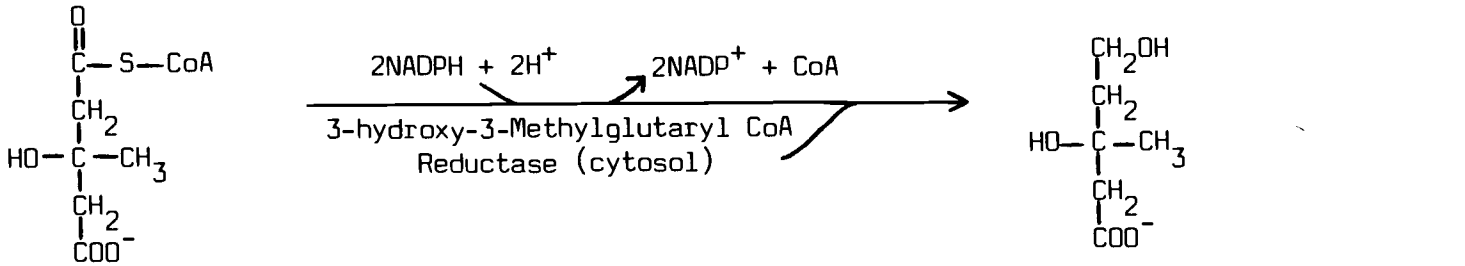
APPENDICES

APPENDIX A. The synthesis of cholesterol from Acetyl CoA including the steroid hormones produced (Stryer, 1981).



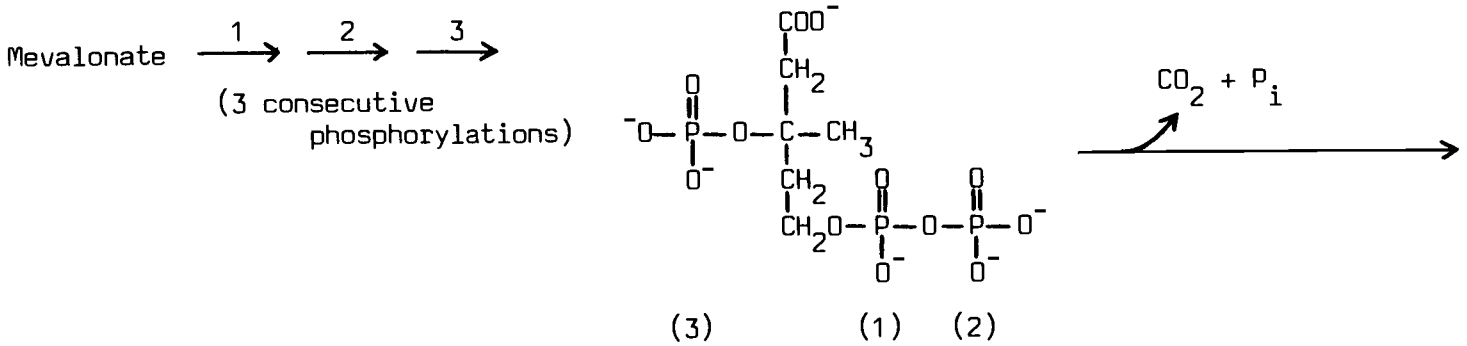
Acetyl CoA
from Citric Acid Cycle

Acetoacetyl CoA

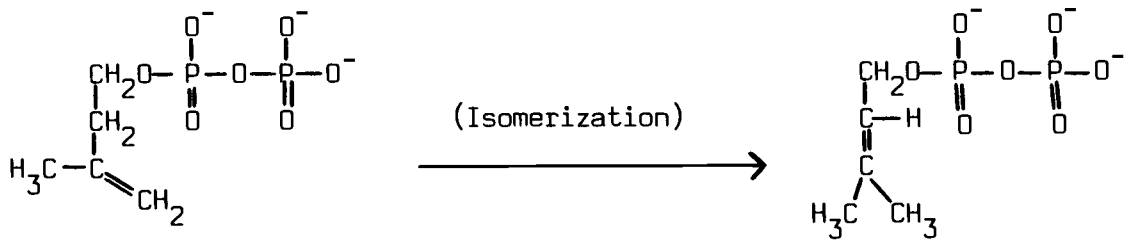


3-Hydroxy-3-Methylglutaryl CoA

Mevalonate-the committed step
in cholesterol synthesis



3-Phospho-5-Pyrophospho-Mevalonate
(labile intermediate)

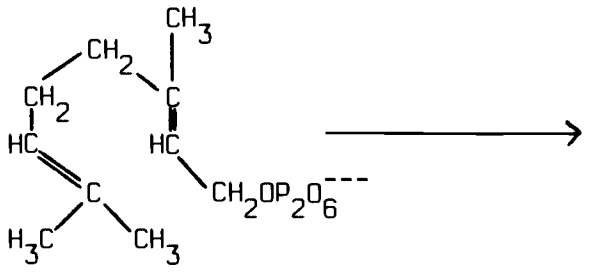


Isopentenyl Pyrophosphate

Dimethylallyl Pyrophosphate (C_5)

Dimethylallyl Pyrophosphate

(condensation with Isopentenyl Pyrophosphate) PP_i

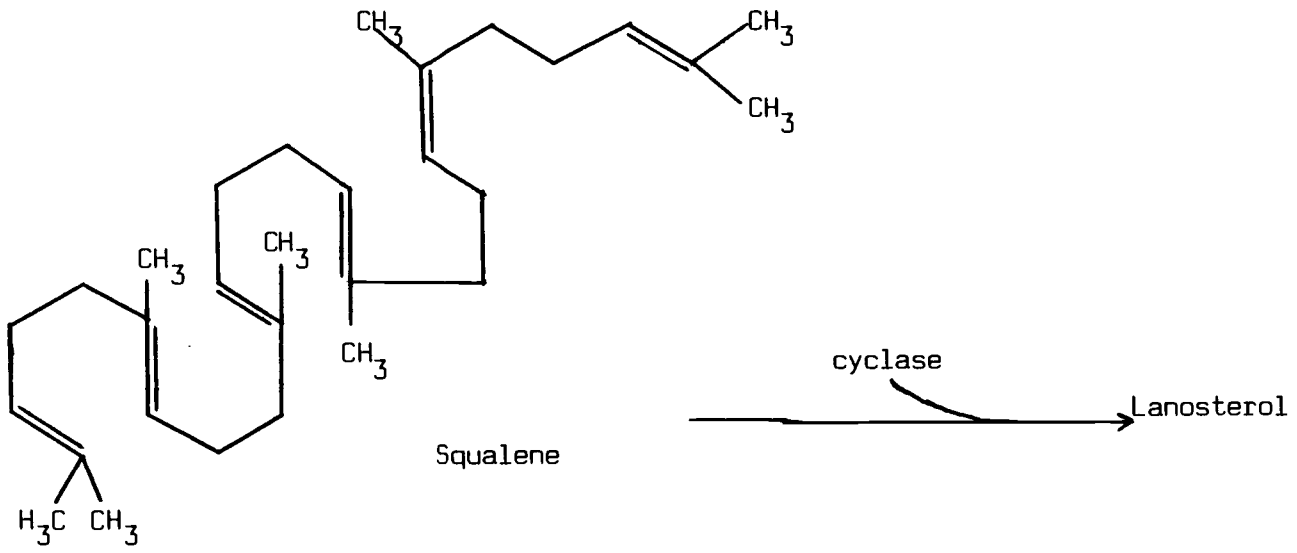


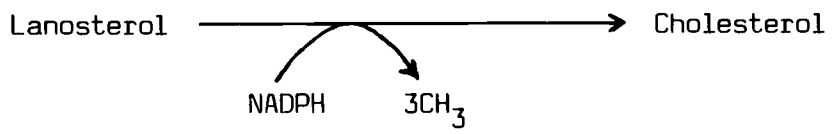
Geranyl Pyrophosphate (C_{10})

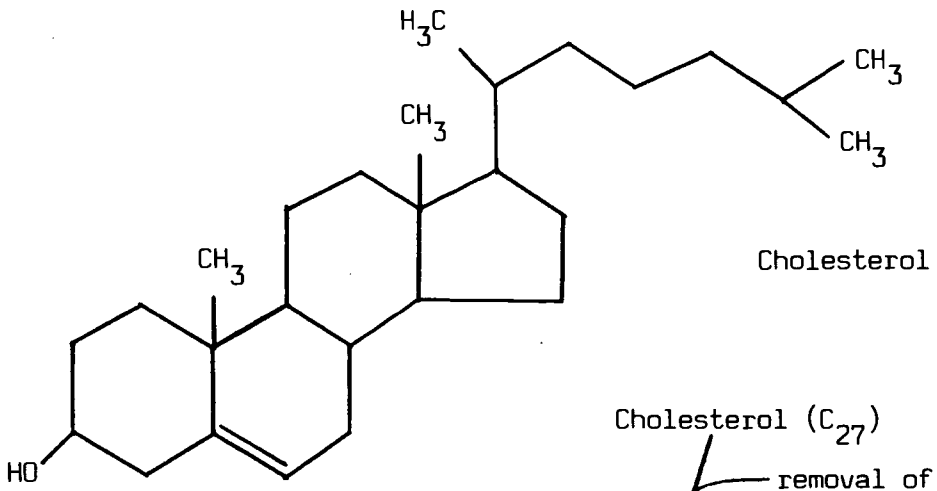
(condensation with Isopentenyl Pyrophosphate) PP_i → Farnesyl Pyrophosphate (C_{15}) NADPH →

$NADP^+ + 2PP_i + H^+$

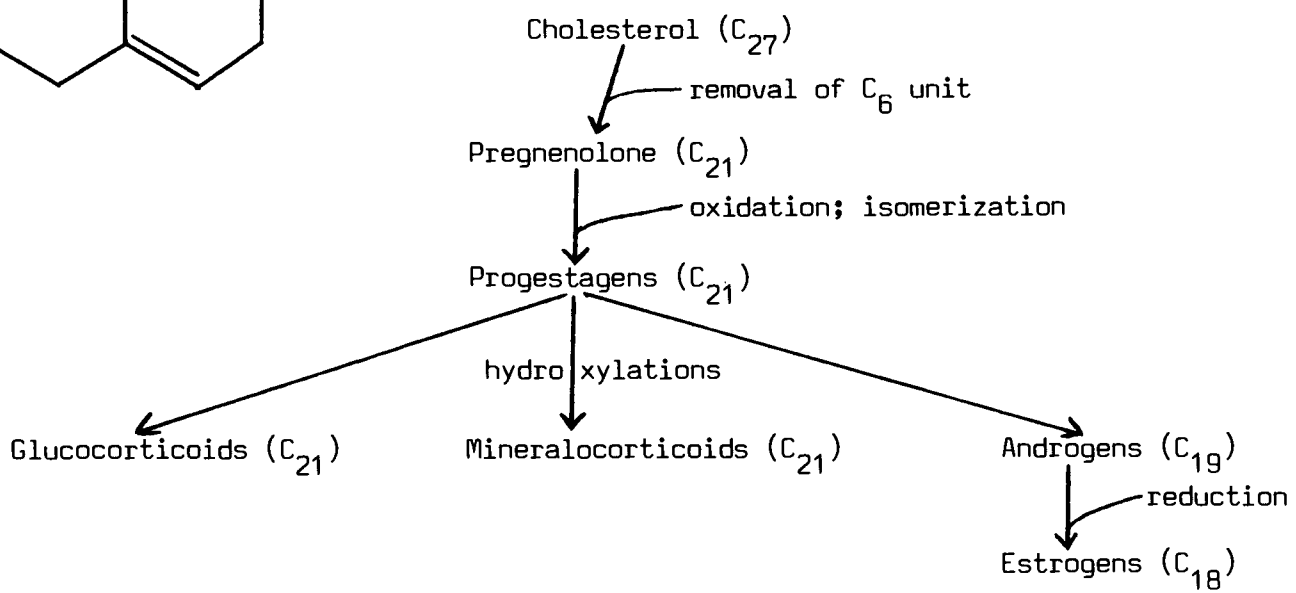
(reductive condensation with Farnesyl Pyrophosphate) → Squalene (C_{30})


CH3
CH3
CH3
CH3
CH3
CH3
H3C
CH3
cyclase
Lanosterol
Squalene


Lanosterol
Cholesterol
NADPH
3CH3



Cholesterol



APPENDIX B. Ration formulations and analysis.

1. Japanese Quail Starter
2. Japanese Quail Developer
3. Japanese Quail Breeder

JAPANESE QUAIL STARTER--RATION #1876

INGREDIENT	%
Yellow Corn	47.00
Soybean Meal (47.5%)	41.40
Fish Meal - Herring	2.50
Alfalfa Meal, dehy. (17%)	3.00
Meat and Bone Meal	5.00
Defluorinated Phosphate	0.50
Salt, iodized	0.25
Trace Mineral Mix[1]	0.05
Vitamin Premix[2]	0.20
D,L Methionine (98%)	0.10
TOTAL	100.00

[1]Trace Mineral Mix provides per kg of ration: Ca, 97.5mg; Mn, 60mg; Fe, 20mg; Cu, 2mg; I, 1.2mg; Zn, 27.5mg

[2]Vitamin Premix provides per kg of ration: Vit. A, 3304IU; Vit. D, 1111ICU; Riboflavin, 3.3mg; d-Pantothenic acid, 5.51mg; Niacin, 22.01mg; Choline, 191mg; Vit. B_{12}, 5.51mcg; Vit. E, 1.1IU; Vit. K, 0.55mg; Folacin, 0.22mg

NUTRIENT	ANALYSIS
Protein	28.65%
Fat	3.16%
Linoleic Acid	1.04%
Fiber	2.97%
Met. Energy	2812.27KCAL/KG
Calcium	0.97%
Total Phos.	0.84%
Avail Phos.	0.55%
Manganese	83.61MG/KG
Sodium	0.19%
Iron	93.42MG/KG
Copper	18.92MG/KG
Iodine	46.45MG/KG
Potassium	1.16%
Magnesuim	1397.50MG/KG
Zinc	59.56MG/KG
Chlorine	0.28%
Selenium	0.13MG/KG
Sulfur	1008.18MG/KG
Xanthophyll	18.95MG/KG
Vitamin A	10201.00IU/KG
Vitamin D	1100.00ICU/KG
Riboflavin	5.89MG/KG
D-Pant. Acid	14.28MG/KG
Niacin (Total)	47.25MG/KG
Niacin (Avail)	38.55MG/KG
Choline	1832.72MG/KG
Betaine	105.60MG/KG
Vitamin B12	15.93MCG/KG
Vitamin E	23.34IU/KG
Vitamin K	0.81MG/KG
Pyridoxine	5.15MG/KG
Folacin	2.07MG/KG
Biotin (Total)	0.20MG/KG
Biotin (Avail)	0.19MG/KG
Thiamine	2.85MG/KG
Arginine	1.95%
Glycine	1.84%
Histidine	0.66%
Isoleucine	1.40%
Leucine	2.34%
Lysine	1.69%
Methionine	0.56%
Cystine	0.47%
Phenylalanine	1.48%
Tyrosine	1.07%
Threonine	1.16%
Valine	1.49%
Tryptophan	0.34%
Serine	1.53%

JAPANESE QUAIL DEVELOPER
BROILER STARTER--RATION #1476

INGREDIENT	%
Yellow Corn	64.35
Soybean Meal (44%)	26.00
Meat and Bone Meal	5.00
Alfalfa Meal, dehy. (17%)	2.50
Limestone Flour	1.00
Defluorinated Phosphate	0.50
Salt, iodized	0.30
Trace Mineral Mix[1]	0.05
Vitamin Premix[2]	0.20
MHA (80%)	0.10
TOTAL	100.00

[1]Trace Mineral Mix provides per kg of ration: Ca, 97.5mg; Mn, 60mg; Fe, 20mg; Cu, 2mg; I, 1.2mg; Zn, 27.5mg

[2]Vitamin Premix provides per kg of ration: Vit. A, 3304IU; Vit. D, 1111ICU; Riboflavin, 3.3mg; d-Pantothenic acid, 5.51mg; Niacin, 22.01mg; Choline, 191mg; Vit. B_{12}, 5.51mcg; Vit. E, 1.1IU; Vit. K, 0.55mg; Folacin, 0.22mg

NUTRIENT	ANALYSIS
Protein	20.34%
Fat	3.44%
Linoleic Acid	1.29%
Fiber	3.34%
Met. Energy	2910.67KCAL/KG
Calcium	1.25%
Total Phos.	0.75%
Avail Phos.	0.49%
Manganese	74.73MG/KG
Sodium	0.20%
Iron	110.59MG/KG
Copper	8.02MG/KG
Iodine	39.00MG/KG
Potassium	0.82%
Magnesuim	2425.11MG/KG
Zinc	46.61MG/KG
Chlorine	0.26%
Selenium	0.08MG/KG
Sulfur	2130.85MG/KG
Xanthophyll	22.08MG/KG
Vitamin A	9885.15IU/KG
Vitamin D	1100.00ICU/KG
Riboflavin	5.30MG/KG
D-Pant. Acid	13.01MG/KG
Niacin (Total)	46.59MG/KG
Niacin (Avail)	36.68MG/KG
Choline	1397.27MG/KG
Betaine	88.00MG/KG
Vitamin B12	10.40MCG/KG
Vitamin E	25.09IU/KG
Vitamin K	0.77MG/KG
Pyridoxine	4.31MG/KG
Folacin	0.68MG/KG
Biotin (Total)	0.15MG/KG
Biotin (Avail)	0.15MG/KG
Thiamine	4.12MG/KG
Arginine	1.40%
Glycine	1.32%
Histidine	0.52%
Isoleucine	1.01%
Leucine	1.89%
Lysine	1.11%
Methionine	0.39%
Cystine	0.34%
Phenylalanine	1.08%
Tyrosine	0.68%
Threonine	0.88%
Valine	1.04%
Tryptophan	0.24%
Serine	1.08%

JAPANESE QUAIL BREEDER--RATION #1771

INGREDIENT	%
Yellow Corn	55.20
Soybean Meal (47.5%)	29.60
Fish Meal - Herring	3.00
Alfalfa Meal, dehy. (17%)	2.50
Limestone Flour	3.90
Defluorinated Phosphate	0.30
Salt, iodized	0.25
Trace Mineral Mix[1]	0.05
Vitamin Premix[2]	0.20
Meat and Bone Meal	5.00
TOTAL	100.00

[1]Trace Mineral Mix provides per kg of ration: Ca, 97.5mg; Mn, 60mg; Fe, 20mg; Cu, 2mg; I, 1.2mg; Zn, 27.5mg

[2]Vitamin Premix provides per kg of ration: Vit. A, 3304IU; Vit. D, 1111ICU; Riboflavin, 3.3mg; d-Pantothenic acid, 5.51mg; Niacin, 22.01mg; Choline, 191mg; Vit. B_{12}, 5.51mcg; Vit. E, 1.1IU; Vit. K, 0.55mg; Folacin, 0.22mg

NUTRIENT	ANALYSIS
Protein	24.05%
Fat	3.38%
Linoleic Acid	1.14%
Fiber	2.68%
Met. Energy	2845.40KCAL/KG
Calcium	2.35%
Total Phos.	0.75%
Avail Phos.	0.50%
Manganese	88.27MG/KG
Sodium	0.18%
Iron	181.87MG/KG
Copper	4.11MG/KG
Iodine	38.95MG/KG
Potassium	0.93%
Magnesuim	1958.75MG/KG
Zinc	55.45MG/KG
Chlorine	0.28%
Selenium	0.14MG/KG
Sulfur	1122.20MG/KG
Xanthophyll	19.80MG/KG
Vitamin A	9515.80IU/KG
Vitamin D	1100.00ICU/KG
Riboflavin	5.59MG/KG
D-Pant. Acid	12.91MG/KG
Niacin (Total)	46.71MG/KG
Niacin (Avail)	36.46MG/KG
Choline	1564.21MG/KG
Betaine	88.00MG/KG
Vitamin B12	17.03MCG/KG
Vitamin E	24.55IU/KG
Vitamin K	0.77MG/KG
Pyridoxine	4.44MG/KG
Folacin	1.65MG/KG
Biotin (Total)	0.17MG/KG
Biotin (Avail)	0.16MG/KG
Thiamine	2.82MG/KG
Arginine	1.61%
Glycine	1.57%
Histidine	0.55%
Isoleucine	1.16%
Leucine	2.02%
Lysine	1.37%
Methionine	0.41%
Cystine	0.39%
Phenylalanine	1.23%
Tyrosine	0.88%
Threonine	0.98%
Valine	1.23%
Tryptophan	0.28%
Serine	1.26%

APPENDIX C. Production data from parent generation to third generation

a = blood sampled at 4 weeks of age
b = 6 week period beginning when birds were 7 weeks of age
c = from hatch to 6 weeks of age

PRODUCTION PARAMETERS - PARENT GENERATION

Line	Avg. mg/dL Cholesterol[a]	%Prod[b]	%Fert	%HFE
C	155.4	73.6	94.9	90.8
L1	120.2	70.0	79.2	84.0
L2	125.9	71.0	87.4	73.1
H1	196.8	66.8	76.9	67.0
H2	198.4	76.2	88.0	82.6

PRODUCTION PARAMETERS - FIRST GENERATION

Line	Avg. mg/dL Cholesterol[a]	%Prod[b]	%Fert	%HFE	%Mort[c]
C	146.8	78.1	84.2	90.0	25.0
L1	111.8	77.6	83.7	76.1	32.5
L2	128.1	70.9	92.3	81.6	17.7
H1	155.7	75.7	84.0	74.4	9.7
H2	178.7	79.9	95.3	85.3	33.3

PRODUCTION PARAMETERS - SECOND GENERATION

Line	Avg. mg/dL Cholesterol[a]	%Prod[b]	%Fert	%HFE	%Mort[c]
C	132.7	76.8	88.9	69.4	25.5
L1	108.1	82.0	86.3	81.3	27.0
L2	118.5	75.8	100.0	91.3	37.5
H1	169.8	81.6	91.9	74.6	17.7
H2	194.9	82.4	95.8	91.4	25.0

PRODUCTION PARAMETERS - THIRD GENERATION

Line	Avg. mg/dL Cholesterol[a]	%Prod[b]	%Fert	%HFE	%Mort[c]
C	97.2	76.5	84.9	83.4	44.7
L1	91.1	79.0	98.6	79.7	34.7
L2	93.3	72.7	92.9	75.5	22.2
H1	123.7	74.4	86.5	63.0	21.1
H2	140.6	67.6	87.2	82.9	28.9