EFFECTS OF SUBCUTANEOUS INJECTIONS OF RAW EGG-WHITE UPON PERFORMANCE OF GROWING SWINE AND CHICKENS

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BENG TATT OH
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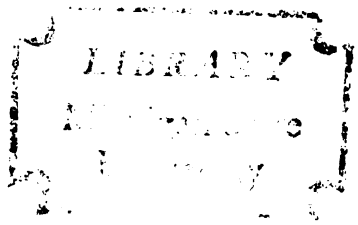
THESIS



ABSTRACT

EFFECTS OF SUBCUTANEOUS INJECTIONS OF
RAW EGG-WHITE UPON PERFORMANCE OF
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By

BENG TATT OH

Attempts to stimulate the growth rate of swine by using chicken egg-white injections were unsuccessful. A total of 66 pigs divided into three trials were used and the pigs were injected subcutaneously with the following dosages of chicken egg albumen: 15 ml, 20 ml, 25 ml, 30 ml, 40 ml, 50 ml, and 55 ml. Injectins were made at weekly intervals for four weeks with increased dosage each week.

Injected pigs failed to show any improved body weight gain. In the second trial the treated pigs which received 15 ml, 20 ml, 25 ml, and 30 ml of chicken egg-white injections had better feed efficiency than did controls. The feed conversion for the treated group was 3.3 kg of feed to 1 kg gain in weight and for the control 4.2 kg of feed to 1 kg gain in weight. No noticeable toxicity symptom was found in the pigs.

When the dosages were increased to 40 ml, 50 ml, or 55 ml in Trial III depressed appetite, vomiting and loose stool

were noticed in the treated pigs. Therefore, dosage above 40 ml may produce some toxicity which also reduce the rate of growth.

Weekly subcutaneous injection of chicken egg albumen at ½ ml, 1 ml, 1½ ml, 2 ml levels per week for four weeks to 92 growing chicks did not produce any significant gain in body weight. Female chickesn showed slight response to the chicken albumen injection but statistically the gain in weight was not significant.

A total of 40 hens, divided into four treatments, namely quail egg albumen (QEA), chicken egg albumen (CEA), Ringer's solution (RS), and control (CONT) were conducted. During 107 days of egg production QEA treated hens produced 906 eggs, control hens 803 eggs, CEA treated hens 797 eggs, and RS treated hens 779 eggs. Hen housed production percentage was 84.7%, 75.1%, 74.5% and 72.8% for QEA, CONT., CEA, AND RS, respectively. The QEA treated hens had a significantly higher ($P < 0.01$) egg production than those in the other groups. During the five weeks of treatment there was no significant difference among the groups in their egg production. The QEA group started to lay more eggs three weeks after termination of treatment and the CEA group was laying slightly less than QEA group for only three weeks, then dropped to about control level. Average egg size for these groups was QEA 57.4 gm, RS 58.6 gm, CONT 59.6 gm and CEA 60.6 gm.

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Beng Tatt Oh

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I. INTRODUCTION

What is in the egg albumen which can stimulate growth in pigs? Can there be an unidentified growth factor in egg albumen? Can a small amount (20 to 40 ml) of chicken egg albumen, 88.5% of which is water injected into a pig subcutaneously each week be enough to balance the amino acids requirement, thus resulting in improved growth rate?

In 1964 while the author was a manager of a pig farm, he injected egg albumen into pigs subcutaneously hoping to stimulate growth rate of pigs. He obtained very good response in his treated groups as compared to controls with regards to growth rate. His assistant continued with the albumen treatments on pigs four weeks before selling long after the author had left the farm to join the College of Agriculture, Malaya. Later this assistant left the farm to become a farm manager for another person. He developed the farm from 150 pigs to 4,000 pigs within five years. He stopped injecting the pigs with chicken egg albumen after an incident in 1969 where 60% of the 90 injected pigs showed severe symptoms of toxicity and this man was quick enough to save them by injecting Vitamin C plus 5% glucose.

The author managed to convince his professor, Dr. T. H. Coleman into allowing him to pursue the experiment further

with better supervision and control. Dr. E. R. Miller of Animal Husbandry Department consented to assist him in the experiment with the pigs at MSU Swine Research Farm.

The chicken egg-white was also injected subcutaneously to growing chickens to determine the effects on their subsequent growth. Japanese quail egg albumen was also introduced into the laying hens to determine the effects on egg production.

The author had searched through the literature and could not find any article of farm animals being injected with egg albumen. There are reports of laboratory rats showing symptoms of toxicity, depressed feed intake and retarded growth upon treatment of egg-white orally or by injection.

Since no work with egg albumen subcutaneous injection on farm animals has been reported this study was initiated (I) to repeat the experiment which was conducted in Malaysia a decade ago, and (II) to determine the effects of quail egg and chicken egg albumen injections upon the performance of growing chickens and layers.

II. OBJECTIVES

The specific objectives of this research were as follows:

1. To determine the effects of subcutaneous injections of albumen in growing swine on subsequent growth rate and/or efficiency of feed utilization.

2. To determine the effects of subcutaneous injection of chicken egg-white and quail egg-white in hens upon their egg production.

3. To explore the possible new uses of egg albumen of chicken and quail thereby creating a new market for quail and chicken eggs.

III. REVIEW OF LITERATURE

Introduction

The proteins of chicken egg-white are particularly good systems for study because they have unique biochemical activities, are relatively easily available and exist in homologous forms, not only in similar organs or fluids of different species but also in different organs or fluids of other species (Feeney, 1964). Eggs have long been recognized for their most excellent essential amino acid pattern and the high biological value of the protein. Only fish protein ranks in a class with eggs, but egg protein is superior as a source of the amino acids required by man (Scott *et. al.*, 1969).

Albumen may be regarded as a protein system consisting of ovomucin fibers in an aqueous solution of numerous globular proteins. Since an egg is a complex biological system, it is a convenient and challenging material for us to explore. Many fundamental studies have been made on its protein and lipid components. Several excellent review articles have been written by Baker (1968), Feeney (1964), Parkinson (1966), Warner (1954), and Feeney and Allison (1969).

Composition of a Chicken Egg

The egg has been well studied by many investigators, for example: Romanoff and Romanoff, 1949; Bellairs et. al., 1964; Williams, 1967; Shenstone, 1968; Baker, 1968; Cook, 1968; Feeney and Allison, 1969; King, 1972. The composition of the "ideal" egg or the limits for many substances within which normal growth of the embryo can take place, is still not known (Warren and Conrad, 1939; Lacassagne, 1960; Shenstone, 1968; Bacon and Cherms, 1968; Bacon and Skala, 1968; Gilbert, 1970).

The general values given in Tables 1 to 4 describe the "typical egg" and are based on data given by Romanoff and Romanoff (1949), Brooks and Taylor (1955), Cunningham et. al. (1960), Cotterill et. al. (1962), Marion et. al. (1966), Jaffe' (1964), Chung and Stadelman (1965), Kline et. al. (1965), Hill et. al. (1966) and Shenstone (1968), although wide variations are possible.

In basic terms, the egg has three distinct fractions; "yolk" (ovum), "white" (albumen) and "shell" (calcified exterior, cuticle and membranes), (Fig. 1). In Table 5, the egg is taken to consist of ovum, albumen, shell membranes, shell and cuticle.

Factors affecting egg composition are numerous and these include breed, age of the hen, position of the egg in a sequence, rate of lay, time of year, ambient temperature, food quality and quantity, noise and disease (Gilbert, 1971).

TABLE 1

AVERAGE CHARACTERS OF CHICKEN EGG

Weight	58 g.
Long Axis	5.7 cm
Short Axis	4.2 cm
Long Circumference	15.7 cm
Short Circumference	13.5 cm
Volume	53.0 cm^3
Gross Surface Area[a]	68.0 cm^2

[a]True surface area may be many times larger because there are many small irregularities and indentations.

TABLE 2

RELATIVE COMPOSTIION OF CHICKEN EGG: WATER AND SOLIDS OF SHELL, YOLK AND WHITE AS PERCENTAGE OF WHOLE EGG

	Percentage of Whole Egg	Water Percentage of Total Egg	Solid Percentage of Total Egg
White	58.0	76.2	20.0
Yolk	32.0	23.6	51.0
Shell	10.0	0.2	29.0

TABLE 3

CORRESPONDING WEIGHTS OF THE VARIOUS COMPONENTS OF CHICKEN EGG (g)

Component	Solid	Water	Total
White	3.8	29.4	33.2
Yolk	9.9	9.1	19.0
Shell	5.7	0.1	5.8
Egg	19.4	38.6	58.0

TABLE 4

PERCENTAGE COMPOSITION OF THE SHELL, WHITE AND YOLK

Chemical Class	Shell	White	Yolk
Water	1.0	88.5	47.5
Protein	4.0	10.5	17.4
Lipid	-	-	33.0
Carbohydrate	Some in Protein	0.5	0.2
Inorganic Ions	95.0	0.5	1.1
Other	-	-	0.8

TABLE 5

EGG COMPONENTS

Common Name	Specific Name	Components
Yolk	Ovum	(a) Blastodisc (infertile) or Blastoderm (fertile)
		(b) Nucleus of Pander
		(c) Latebra ("white"yolk)
		(d) Yolk ("yellow" yolk)
		(e) "Vitelline" Membranes
		(1) Primary membrane, the true Vitellin membrane
		(2) Secondary membrane, the Perivitellin membrane
		(3) 2 tertiary membranes, formed from the oviduct
White	Albumen	(a) "Albumen Ligaments"
		(b) Chalazae
		(c) Chalaziferous Region
		(d) Inner Thin White
		(e) Middle Thick White
		(f) Outer Thin White
Shell	Shell Membranes	(a) Inner Membrane (air space)
		(b) Outer Membrane
	Shell Cuticle	(c) Organic Matrix
		(d) Inorganic Crystals

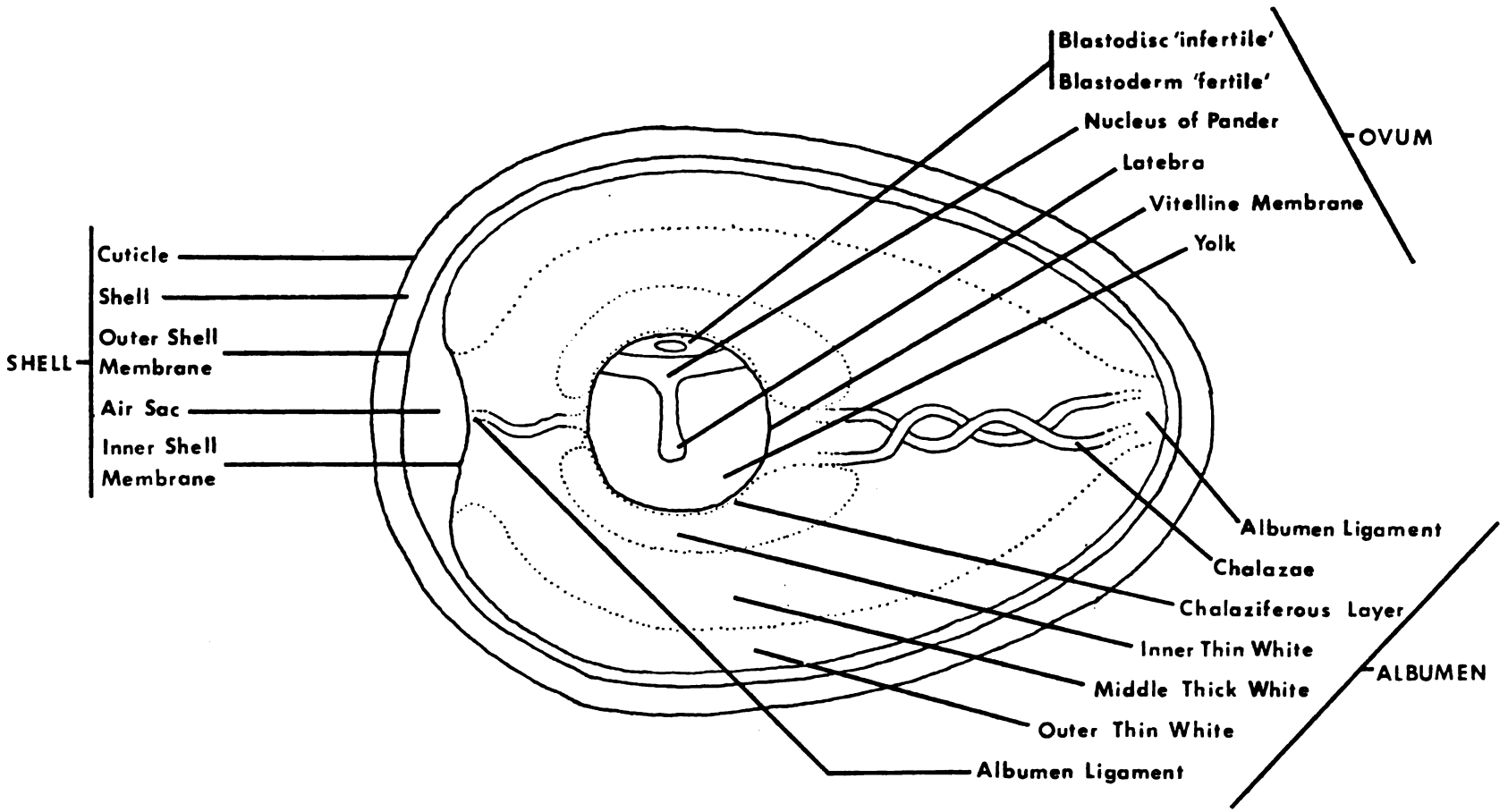

Fig. 1. Diagram of the hens egg.

Composition of Egg Albumen

The egg albumen consists shiefly of water, of which it is the developing embryo's principal reservoir. The albumen's main organic constituent is protein; other components are small amounts of carbohydrates and minerals and trace amounts of lipids. These amounts are shown in Table 6.

TABLE 6

COMPOSITION OF EGG ALBUMEN

Total Egg Albumen	Amounts (gram)
Total	32.9
Water	28.9
Solids	4.0
Organic Matter	3.8
Proteins	3.5
Lipids	trace
Carbohydrates	0.3
Inorganic Matter	0.2

Source: Romanoff & Romanoff (1949)

The whites of eggs laid by a single bird are usually fairly uniform in their proportions of water and solids. On the other hand, the average percentages of the various

constituents of the albumen are much the same in the eggs of all species of precocial birds. Table 7 shows the proportional amounts of major chemical constituents in the egg albumen of various birds.

TABLE 7

PROPORTIONAL AMOUNT OF MAJOR CHEMICAL CONSTITUENTS IN EGG ALBUMEN OF VARIOUS BIRDS

Chemical Constituent	Chicken (32.9 gm)* (%)	Turkey (44.2 gm)* (%)	Guinea Fowl (19.9 gm)* (%)	Duck (40.4 gm)* (%)	Goose (110.2 gm)* (%)
Water	87.8	86.5	86.6	86.8	86.7
Solids	12.1	13.5	13.4	13.2	13.3
Organic Matter	11.5	12.8	12.6	12.4	12.5
Proteins	10.6	11.5	11.6	11.3	11.3
Fats (Lipids)	0.03	0.03	0.03	0.08	0.04
Carbohydrates	0.9	1.3	1.0	1.0	1.2
Inorganic Matter	0.6	0.7	0.8	0.8	0.8

*Indicate the average weight of egg albumen of each species.

Source: Romanoff & Romanoff (1949)

As indicated in the table above there is a great similarity in albumen from eggs of the species shown and this may explain why it is possible, in the laboratory, to substitute duck for chicken albumen without seriously interferring with early embryonic development (Loisel, 1900; Romanoff and Romanoff, 1949).

Various Layers of Albumen

Egg-white is essentially an aqueous solution of proteins. Four distinct layers can be recognized.

(1) a fluid "outer thin" layer (Outer Liquid)

(2) a firm "thick" layer (Middle Dense)

(3) another fluid layer (Inner Liquid)

(4) a shallow dense layer (Chalaziferous)

The percentage of water in the four layers varies in different species as shown in Table 8.

These layers differ in chemical and physical properties; in particular, the concentration of ovomucin is much greater in the "thick" layer than in the "thin" ones. The proportions of the different layers vary widely, but it has been established that the percentage of the thick white is characteristic of the individual (Parkinson, 1966), (see Table 9). Table 10 shows the concentration of the major elements in albumen of egg.

Earlier work on egg proteins has been comprehensively reviewed by Fevold (1951) and by Warner (1954) and later work

TABLE 8

PERCENTAGE OF WATER IN THE FOUR LAYERS OF ALBUMEN IN VARIOUS BIRDS

Species	Outer Liquid	Middle Dense	Inner Liquid	Chalaziferous
Chicken	88.8	87.6	86.4	84.3
Pheasant	89.0	88.0	86.3	85.3
Quail	88.1	87.2	85.8	84.9
Duck	87.4	86.7	85.8	84.3
Average	88.3	87.4	86.1	84.7

Investigators: Romanoff (1929, 1943), Almquist and Lorenz (1933)

TABLE 9

PROPORTION AND SOLID (PROTEIN) CONTENT OF THE VARIOUS COMPONENTS OF CHICKEN EGG ALBUMEN

Component	Weight (g)	Percentage of Total Albumen	Percentage Solids in Each Component
Outer Thin Layer	7.7	23.0	11.2
Thick Layer	18.9	57.0	12.4
Inner Thin Layer	5.6	17.0	13.6
Chalaziferous Layer	0.9	0.2	15.7
Chalaza 1	0.9	0.2	15.5
Chalaza 2	0.9	0.8	15.5

From the data of Almquist and Lorenz (1933), Romanoff and Romanoff (1949) and Besch and Sluka (1966).

TABLE 10

CONCENTRATION OF THE MAJOR ELEMENTS IN ALBUMEN OF THE LAID EGG

(Calculated on the basis of the weight of the albumen being 33.2 g.)

Element	mg/total albumen
Sodium	48.8
Potassium	46.5
Calcium	4.3
Magnesium	3.3
Iron	.003
Sulphur	65.0
Chloride	42.2
Phosphate (as P)	3.7

Recalculated from the collated data of Shenstone (1968).

has been summarized by Feeney (1964), Parkinson (1966), Baker (1968) and Feeney and Allison (1969); a collated summary of their data is given in Table 11.

TABLE 11

REPRESENTATIVE COMPOSITION OF EGG-WHITE SOLIDS

(Calculated on the basis of the egg weighing 58 g.)

	Percentage of Total Solids
Ovalbumen	54.0
Ovotransferrin (conalbumen)	13.0
Ovomucoid	11.0
Ovomucin	1.5 - 2.9
Lysozyme (G_1 - globulin)	3.5
G_2 - globulin	4.0
G_3 - globulin	4.0
Ovomacroglobulin	0.5
Ovoglycoprotein	0.5 - 1.0
Flavoprotein - apoprotein	0.8
Ovoinhibitor	0.1 - 1.5
Avidin	0.05

Nutritive Value of Eggs

Eggs are designed by nature to supply all of the nutrients needed in the development of a healthy, sturdy chick. They are a rich source of such high quality protein

that experimental nutritionists often use them as a standard for measuring the quality of other food proteins.

Eggs are also an important source of unsaturated fatty acids (mainly oleic), iron, phosphorus, trace minerals, Vitamins A, E, and K and the B Vitamins, including B 12. As a natural source of Vitamin D, eggs rank second only to fish liver oils. In Table 12 are listed the values of the important nutrients of a fresh egg without shell. The listed value in the table for a specific nutrient in a whole raw egg may vary from the combined values for a raw white and yolk because of differences in analytical technique and source of data.

Many studies have shown that the nutritive value of eggs can vary with the hen's feed and with storage time and conditions. Reports vary as to the effect of diet of the laying bird on composition of eggs produced. Pollard and Carr (1924) and Gerber and Carr (1930) presented evidence indicating that the percentage of protein of pigeon eggs varies with the type of grain fed. Significant differences in protein percentage of eggs of hens on different diets have been reported by Titus, Byerly and Ellis (1933).

Csonka (1950) found that hens on a high protein diet produced eggs with a greater percentage of protein than those on a low protein diet.

In contrast to the above reports several workers (Szorenyl and Ossezehas, 1941; McFarlane et. al., 1930; Calvery and Titus, 1934; and Reder, 1939), have found that

protein content of eggs was not influenced by the diet of the layer.

The general consensus of available reports is that actual grams of protein per egg do not decrease on aging, although evaporation of water and shifts of water from white to yolk sometimes cause changes in percentage of protein (Jenkins et. al., 1920; Mitchell, 1932; Reder, 1939; Romanoff, 1940; Silva, 1947; and Evans and Davidson, 1953).

Significant, but small, genetic differences in nitrogen content of eggs were demonstrated by Arroyave et. al. (1957). Cotterill and Winter (1954) found significant differences among strains in nitrogen content of egg whites from hens of different lineage.

Although the extent that season, environmental temperature, management practices, nutrition, genetics, age of layer and others affect egg quality have been extensively studied, however, satisfactory explanation for these quality variations in relation to differences in composition which affect physical and performance characteristics associated with quality have not been elucidated. Smith et. al. (1954) found that the composition of eggs produced by a hen was an individual characteristic and that changes in egg composition could be associated with laying rate, environmental temperature and age of bird. They reported that higher temperature increased the dry matter, phosphorus, sodium and potassium levels of egg-white.

Cunningham et. al. (1960) indicated that season was highly significant in its effect on the variation of sodium, calcium, and chlorine in egg-white but had little or no effect on potassium, phosphorus, and protein content. They also reported that age of bird was highly significant in its effect on the variation of phosphorus, chlorine, and protein in egg-white; affected the calcium content to a lesser degree; and had no effect on the sodium and potassium content. May and Stadelman (1960) found that the strain of hen significantly influenced percentage of moisture, protein of fresh eggs and protein of dried egg. They also reported that age of hen and season significantly influenced egg contents, weight, albumen height, Haugh units, grams of protein per egg and in one case, percentage moisture and percentage protein of dried egg.

The values of the composition of fresh shell eggs reported in Table 12 and Table 13 were obtained directly from Dr. Owen J. Cotterill of the University of Missouri-Columbia, Mo., who had kindly granted the author the permission to use them here.

Biochemistry of Egg-White Proteins

Egg white has been shown to contain about 40 individual proteins. Of these 24 or so are minor proteins which have been found on starch-gel electrophoretograms. In the main, these minor components of egg-white remain uncharacterized,

TABLE 12

COMPOSITION OF FRESH SHELL EGGS

(100 g. Liquid Basis)

	Whole	White	Yolk
GROSS COMPOSITION			
Solids[a] - g.	25.28	10.72	50.78
Protein[b] (N x 6.25) - g.	12.03	10.07	16.16
Lipids[c] (total) - g.	12.31	-	34.10
Saturated	5.57	-	13.35
Monounsaturated	7.03	-	17.02
Polyunsaturated	2.19	-	5.23
Ash[a] (total) - g.	.98	.69	1.65
AMINO ACIDS[b]			
Alanine - g.	.644	.580	.793
Arginine - g.	.771	.560	1.137
Aspartic acid - g.	1.197	1.090	1.302
Cystine - g.	.274	.298	.262
Glutamic acid - g.	1.487	1.320	1.817
Glycine - g.	.393	.330	.479
Histidine - g.	.279	.212	.396
Isoleucine - g.	.600	.510	.808
Leucine - g.	.998	.828	1.384
Lysine - g.	.851	.660	1.204
Methionine - g.	.388	.388	.376
Phenylalanine - g.	.572	.550	.618
Proline - g.	.548	.450	.643
Serine - g.	.921	.636	1.297
Threonine - g.	.597	.422	.808
Tryptophane - g.[e]	.173	.148	.237
Tyrosine - g.	.528	.408	.726
Valine - g.	.781	.665	.942
FATTY ACIDS[c] (Goldfisch: chloroform—methanol extraction)			
Caprylic (8:0) - g.	.056	-	.152
Capric (10:0) - g.	.014	-	.022
Lauric (12:0) - g.	.009	-	.019
Myristic (14:0) - g.	.053	-	.160
Myristoleic (14:1) - g.	.018	-	.045

TABLE 12 (Con't.)

	Whole	White	Yolk
Palmitic (16:0) - g.	3.944	-	9.332
Palmitoleic (16:1) - g.	.625	-	1.432
Stearic (18:0) - g.	1.196	-	2.877
Oleic (18:1) - g.	6.388	-	15.541
Linoleic (18:2) - g.	2.056	-	4.908
Linolenic (18:3) - g.	.050	-	.106
Arachidic (20:0) - g.	.075	-	.167
Arachidonic (20:4) - g.	.083	-	.220
Behenic (22:0) - g.	.222	-	.615
VITAMINS			
Retinol - ug.	124.0	-	228.2
D - IU	3.62	-	16.98
E - mg.	1.014	-	3.92
B_{12} - mcg.	1.08	.02	3.16
Biotin - mcg.	18.3	5.1	40.8
Choline - mg.	824.1	1.25	1109.1
Folic acid - mg.	.293	.001	.673
Inositol - mg.	15.46	3.73	33.97
Niacin - mg.	.089	.094	.059
Pantothenic acid - mg.	1.376	.127	4.904
Pyridoxine - mg.	.137	.005	.349
Riboflavin - mg.	.320	.253	.457
Thiamine - mg.	.089	.003	.253
MINERALS			
Calcium - mg.	58.45	8.61	136.39
Chlorine - mg.	172.1	175.5	165.5
Copper - mg.	.062	.023	.132
Iodine - mg.	.072	.0074	.167
Iron - mg.	2.25	.011	5.92
Magnesium - mg.	12.41	12.44	12.35
Manganese - mg.	.0413	.0057	.1132
Phosphorus - mg.	237.94	14.26	607.34
Potassium - mg.	138.0	147.2	110.1
Sodium - mg.	139.14	183.44	60.73
Sulfur - mg.	165.5	158.4	165.5
Zinc - mg.	1.50	.01	5.76

Source: O. J. Cotterill et. al., 1973.

TABLE 13

CONVERSION FACTORS FOR EGG SAMPLES

	Whole Egg	White	Yolk
Yield:			
Based on total egg*	87.2%	54.8%	32.4%
Based on liquid only	-	62.9%	37.1%
Amount/egg (g.)	53.0	33.3	19.7**
Solids Level:			
Liquid	25.28%	10.72%	50.78%
Freeze-dried	97.75%	94.67%	98.66%

*Total egg weight was 60.76 g.

**Includes adhering albumen.

Source: O. J. Cotterill et. al., 1973.

and not all are always present (Gilbert, 1971).

From starch-gel electrophoresis, Lush (1961) distinguished 19 minor components of fowl's egg-white, some undoubtedly represent slight genetical and/or chemical heterogeneity of the 12 major types. These are, in the order of concentrations in the albumen as in Table 14; which shows a summary of characteristics of egg-white proteins from the hen (data were taken from Baker, 1968, and Feeney and Allison, 1969.

Several of the egg-white proteins are known in homogeneous form (Warner, 1954); recent work has extended knowledge of their chemical and physical properties (see Table 14 and 15).

Egg-White Proteins

Avian egg-white is the type of material which is primarily a solution of proteins with a relatively small amount of sugar and salts. This makes the job of protein separation and purification much easier than with many other biological materials. Indeed, egg-white has been the source of several of the standard proteins for biochemists.

Chicken egg-white has been examined by many different electrophoretic techniques.

Electrophoretic studies have shown some of the egg-white proteins to exist in different forms. The first extensive study by this method was that of Longsworth et. al. (1940) using a standard Tiselius type of apparatus and covering the pH range 3.9 - 7.8. They identified eight proteins, including two ovalbumins (A_1 and A_2) and three globulins (G_1, G_2, and G_3) as well as conalbumin, ovomucoid and ovomucin; there was evidence that conalbumin could also exist in two forms (C_1 and C_2). Bain and Deutsch (1947), published electrophoretic patterns at pH 8.6 for the egg-white

TABLE 14

SUMMARY OF CHARACTERISTICS OF EGG-WHITE PROTEINS FROM THE CHICKEN

		%	Isoelectric pH	Approx. Carbohydrate Content %	Molecular Weight	Biological Properties
1.	Ovalbumin	54.0	4.5 - 4.8	3	46,000	
2.	Ovotransferrin	13.0	6.05 - 6.6	2	76,000 to 86,000	Binds iron, copper manganese, zinc; may inhibit bacteria
3.	Ovomucoid	11.0	3.9 - 4.3	22	28,000	Inhibits trypsin
4.	G_2 Globulins	4.0	5.5	?	36,000	
	G_3	4.0	5.8	?	45,000	
5.	Lysozyme	3.5	10.5 - 11.0	?	14,3000 to 17,000	Splits specific B (1-4) D - glucosaminides: lyses bacteria
6.	Ovomucin	1.5 - 2.9	?	19	?	Antiviral haemagglutination
7.	Flavoprotein	0.8	3.9 - 4.1	14	32,000 to 36,000	Binds riboflavin
8.	Ovomacro - Globulin	0.5	4.5 - 4.7	9	760,000 to 900,000	

TABLE 14 (Con't.)

	%	Isoelectric pH	Approx. Carbohydrate Content %	Molecular Weight	Biological Properties
9. Ovoglycoprotein	0.5 - 1.0	3.9	16	24,400	
10. Ovoinhibitor	0.1 - 1.5	5.1 - 5.2	6	44,000 to 49,000	Inhibits proteases, including trypsin and chymotrypsin
11. Avidin	0.05	9.5 - 10.0	8	68,300	Binds biotin
12. Papain Inhibitor	0.1	?	?	12,700	Including papain and ficin

TABLE 15

COMPOSITION OF THE MAJOR EGG-WHITE PROTEINS

	Ovalbumin	Ovotrans-ferrin	Ovomucoid	Lysozyme Res/ 14,307g	Ovomucin	Flavoprotein /Aproprotein	Ovomacro- Globulin	Ovoinhibitor	Avidin
Alanine	5.85	5.09	2.96	12	3.42	2.90	2.81	2.4	2.18
Arginine	3.32	6.07	3.53	11	3.95	2.50	4.06	5.19	7.53
Aspartate	8.18	11.50	13.10	21[a]	8.13	6.70	7.48	8.84	10.15
Cystine	1.34	3.34	6.38	8	3.27	4.80	2.35	4.22	4.04
Glutamate	14.25	11.73	6.85	5[b]	10.64	15.50	10.09	8.07	8.56
Glycine	2.42	3.91	3.28	12	2.56	1.40	1.99	3.11	3.90
Histidine	2.30	2.14	2.10	1	2.00	3.60	1.71	3.16	0.86
Isoleucine	6.22	3.85	1.30	6	4.35	2.30	5.00	3.25	5.48
Leucine	8.28	7.42	4.92	8	6.52	4.90	7.11	4.20	4.96
Lysine	6.02	9.72	6.22	6	5.17	6.40	5.27	5.00	7.04
Methionine	4.74	1.85	.90	2	2.56	3.10	1.88	0.71	1.64
Phenylalanine	6.73	4.98	2.79	3	4.86	3.00	5.08	2.35	6.24
Proline	3.16	3.59	2.67	2	3.73	2.70	3.54	2.74	1.33
Serine	5.24	5.26	3.89	10	5.65	7.30	4.40	3.74	4.76

TABLE 15 (Con't.)

	Ovalbumin	Ovotrans-ferrin	Ovomucoid	Lysozyme Res/ 14,307g	Ovomucin	Flavoprotein /Aproprotein	Ovomacro-Globulin	Ovoinhibitor	Avidin
Threonine	3.03	4.86	5.21	7	4.79	2.30	4.60	5.26	12.33
Tryptophan	1.35	2.86	0.00	6	2.05	4.70	0.93	0.00	5.01
Tyrosine	3.64	4.25	3.91	3	3.87	4.50	4.37	3.51	1.04
Valine	7.18	6.69	5.64	6	5.56	1.60	5.36	3.99	4.40
Sialic Acid	0.00	0.00	0.32	-	4.00	-	0.03	-	-
Hexose	1.76	0.92	9.64	-	7.40	9.20	3.50	3.50	5.22
Glucosamine	1.33	0.97	12.08	-	5.85	5.00	5.20	3.70	3.44
Galactosamine	-	-	-	-	1.31	-	-	-	-

Source: Feeney and Allison (1969).

[a]Includes 13 residues of asparagine.

[b]Includes 3 residues of glutamine.

All values are given as gram of anhydrous amino acid (or carbohydrate) per 100 g protein except for lysozyme. The amino acid sequence for this is known and amino acid content is given as residues/mole.

proteins of thirteen different species of birds. For chicken egg-white their diagrams were similar to those obtained by Longsworth et. al., at pH 7.8, but showed two distinct conalbumin fractions; globulins and ovomucoid were not easily distinguished and formed one heterogeneous region. Forsythe and Foster (1949) used this technique to examine egg-white proteins, other than ovomucin, and found no marked differences in the electrophoretic patterns of outer thin, middle, thick and inner thin layers. The same authors (1950) demonstrated slight but statistically significant differences in the composition of egg-white protein in six genetic strains of chickens. Gordon et. al. (1949), introduced 1% agar gel as a medium for electrophoresis of egg-white proteins at pH values of 6.8 and 8.0, and followed the protein movement by measuring the ultraviolet absorption at 280 mu of 1-cm sections. Their patterns had a broad similarity to the Schbieren diagrams of Longsworth et. al.

Using starch-gel electrophoresis, Lush (1961) established consistent differences between the patterns from eggs from individual hens of different breeds. There appeared to be nineteen separate constituents on some of the electropherograms. Stevens (1962) obtained evidence of sixteen constituents in a similar study and stated that the protein composition of the thick and the inner and outer thin layers of egg-white appeared to be the same. Feeney and co-workers (1963), using the same technique, confirmed the existence of

several uncharacterized minor protein constituents, one of which was isolated by a combination of precipitation and ion-exchange chromatography and characterized as a globulin. Evans and Sandemer (1956), separated egg-white proteins by paper electrophoresis, distinguishing the three forms of ovalbumin.

Rhodes et. al. (1958) obtained the different proteins by ion-exchange chromatography on carboxymethyl-cellulose. Proteins of high purity were obtained in high yields and the method appeared to have several advantages over electrophoresis and the more conventional methods. Mandeles (1960) separated egg-white protein into eleven fractions on a column of diethylaminoethyl cellulose.

Ovalbumin

Ovalbumin can be obtained in crystalline form and with the possible exception of lysozyme, is the most studied egg-white protein. Despite this, it has no known biological function, although it contains all the "essential" amino acids; whether this is important in embryonic nutrition is not known. Ovalbumin, the most abundant protein in egg-white comprises about 54% of the total (see Table 14).

Ovalbumin is the primary protein of chicken egg-white present at four to five times the concentration of the secondary constituents, ovotransferrin and ovomucoid. The properties of egg-white are primarily those of the ovalbumin

with the other constituents contributing mainly to the biological properties of the egg-white. An obvious exception to this is the ovomucin which appears responsible for the high viscosity of thick egg-white. Ovalbumin is a glycoprotein. It has a molecular weight of about 46,000 and an isoelectric pH between 4.5 and 4.8. Other variants of ovalbumin are S-ovalbumin and plakalbumin. S-ovalbumin is a more stable form of ovalbumin and increases during storage of eggs; its properties are similar to ovalbumin, but it possibly arises through alteration of one disulphide bridge (Smith, 1964; Smith and Back, 1965). Plakalbumin has properties very similar to those of ovalbumin (Linderstrom - Lang and Ottesen, 1947; Perlmann, 1952; Lush, 1964; Gournaris and Ottesen, 1965). It is formed by removal of a heptapeptidase by subtilopeptidase A (see Ottesen, 1958) and has a slightly lower molecular weight (43,000) (Linderstrom - Lang and Ottesen, 1947). It migrates more slowly in Tiselius electrophoresis (Lush, 1964). The finding of Yamafuji (1972) that the capacity of egg ovalbumin to bind with and break DNA isolated from spleen may contribute to investigations on the mechanism of antibody formation.

Conalbumin (Ovotransferrin)

Although conalbumin was only identified as the iron-binding protein and anti-bacterial agent in the middle 1940's (Alderton et. al., 1946), it is receiving a relatively

large amount of attention from chemists. Probably the principal reason for the current interest in conalbumin is the fact that it is a homologue of its counterpart in blood serum, serum transferrin (Warner and Weber, 1953). Feeney (1964) referred to avian conalbumin as ovotransferrin. Ovotransferrin comprises about 13 percent of the total protein of egg-white but its precise function is not clear. Its metal-binding properties seem unimportant since less than 1% of its binding potential is used. For embryonic development it is probable that sufficient iron is bound to yolk transferrin although the ability of ovotransferrin in binding copper could be important to the embryo. In the event of bacterial contamination it could compete for metallic ions with the bacterial enzyme systems and thus act as an antibacterial agent. Since it binds copper, its presence may help to preserve the bactericidal properties of lysozyme, which is inactivated by this metal.

Ovotransferrin, a glycoprotein with a molecular weight between 80,000 and 86,000 (Fuller and Briggs, 1956) has an isoelectric pH of about 6.0. It is a single chain (Bezkorevainy et. al., 1968; Feeney and Allison, 1969) with alanine as the N-terminal residue (Williams, 1967). Ovotransferrin can bind other polyvalent metallic ions; in decending order, cobalt - iron - manganese - copper - zinc (Fraenkel - Conrat and Feeney, 1950; Warner and Weber, 1953; Baker, 1967).

The homogeneity of ovotransferrin has been in question (see Stratil, 1967; Baker, 1968; Feeney and Allison, 1969). As Baker (1968) points out, the amount of iron bound (one or more atoms) will affect the physical properties of the complex and thus could lead to resolution of a variety of artefactual "ovotransferrins." Thus in vivo, care must be taken to distinguish between genetical variants of the protein itself and chemical variants resulting from metal-binding. However, Rhodes et. al. (1958) positively identified two forms of ovotransferrin and Lush (1961) and Feeney et. al. (1963a) showed these were in the ratio of 4 to 1. Further work, showing that at least three co-dominant alleles are involved in the coding of ovotransferrin and transferrin (Ogden et. al., 1962; Baker, 1967; Stratil, 1967), also indicated that ovotransferrin is not homogeneous.

Ovomucoid

Ovomucoid was identified as the trypsin inhibitor in egg-white by Lineweaver and Murray (1947). This protein is distinguished by its high content of carbohydrate and its relatively high heat stability. It is a non-heat coagulable glycoprotein. Lewis et. al. (1950) determined its amino acid composition by microbiological methods and drew attention to the absence of free sulphydryl groups (Table 18).

Longsworth et. al. (1940) and Frederica and Deutsch (1949b); concluded independently that at least two

electrophoretically distinct forms exist. Rhodes et. al. (1958) at first found that the ovomucoid fraction obtained from egg-white by chromatography on carboxymethyl-cellulose appeared to be homogeneous when examined by paper electrophoresis. Later, however, refractionation of the preparation of ovomucoid by eluting with buffers of different pH values gave three separate components differing in their contents of sialic acid (Rhodes et. al., 1960). Feeney and Allison (1969) could not find homogeneous ovomucoid. Feeney et. al. (1967) noticed that in all animals, the characteristic of this fraction is its heterogeneity. The major biochemical importance of ovomucoid lies in its power to inhibit proteases. Three proteolytic inhibitors are known in egg-white; the others are ovoinhibitor and a papain-ficin inhibitor. Fowl ovomucoid (also geese and quail) inhibits only trypsin, whereas ovomucoid from other species (turkey, game pheasant, guinea fowl and duck) inhibits both trypsin and chymotrypsin; ovomucoid from yet other birds inhibits only chymotrypsin (Lineweaver and Murray, 1947; Rhodes et. al., 1960; Feeney, 1964). The mechanisms of its action are not understood. However, during the complexing of ovomucoid with trypsin it is possible that important aspects are the electrostatic charges involved, covalent bond(s) and the epsilon groups of the ovomucoid lysine residues. For a full discussion of this subject see Simlot and Feeney (1966) and Feeney and Allison (1969).

Globulins, G_2 and G_3

Longsworth et. al. (1940) showed in egg-white the presence of three globulins termed G_1, G_2 and G_3; Alderton et. al. (1945) subsequently identified G_1 and lysozyme. G_2 and G_3 were further studied by Kaminski (1954) who showed them to be distinct from lysozyme, ovomucoid, ovotransferrin and ovalbumin. Baker and Manwell (1962) identified them on starch-gel electrophoretograms and Feeney et. al. (1963b) were able partially to characterize each fraction.

G_2 and G_3 appear in egg-white in concentrations each of about 4%. G_2-globulin, isolated by a combination of chromatography on DEAE and CM-celluloses and precipitation with ammonium sulphate, was found to have a molecular weight greater than 36,000 but less than 45,000. Isoelectric pH's were estimated as 5.5 for G_2 and 5.8 for G_3. Little other information is available and their functions remain unknown.

Recent biological-genetical work has centered on these proteins. It is known that G_2-globulins are coded or controlled at two alleles (Lush, 1961, 1964; Baker and Manwell, 1962; Cochrane and Annau, 1962; Feeney et. al., 1963b) but further variants of G_2 have been reported (Baker, 1964, 1968).

G_3 is controlled at a single locus with 10 dominant alleles (Lush 1961, 1964; Baker and Manwell, 1962); subsequent investigations have indicated further variants (Baker, 1964, 1968). Baker (1968) suggests that up to 8 variants of

G_3 are present in different strains of hens. The phylogenetical importance of these globulins has been recognized and considerable investigations reported (see Baker, 1968).

Lysozyme (Globulin G_1)

The globulin of egg-white termed G_1 by Longsworth et. al. (1940) was characterized by these workers chiefly by its electrophoretic behavior on examination of diluted egg-white. Further characterization, as well as isolation of G_1, has resulted from research work designed to isolate and characterize a bacteriolytic agent of egg-white. This factor was called lysozyme by Fleming (1922), and has been shown to be identical with G_1 (Alderton et. al., 1945).

(a) Bacteriolytic Properties

Fleming (1922) published a paper entitled, "On A Remarkable Bacteriolytic Agent Occurring in Tissues and Secretions," reporting the detection of lytic agents in tears, sputum, saliva, serum, plasma, leucocytes, eggs and organs of animal body, such as heart, spleen, liver, lungs, and kidneys. The lytic agent was termed lysozyme (lytic enzyme). The highest concentration was found in egg-white, which was active against test organisms in a dilution of 1 to 50,000. That lysozyme is in fact an enzyme, was later demonstrated by Meyer et. al. (1936) and by Epstein and Chain (1940) who showed that it disintegrates the cell walls

of susceptible microorganisms by acting on a specific carbohydrate component of the cell walls. The specific substrate was shown to be present in the cell walls of all susceptible organisms which were investigated but not in those which were resistant to lysozyme (Epstein and Chain, 1940). The susceptibility of various microorganisms to lysozyme has been reported by Fleming (1922), and Waksmann and Woodruff (1942).

(b) Basic Properties

Abraham (1939) described lysozyme as a basic protein containing approximately twenty-two titratable basic groups per mole (m.w. 18,000) as measured by histidine, arginine, and lysine content, and by acid binding at pH 3.0. This value is in good agreement with the data of Fraenkel-Conrat and Cooper (1944) who reported 12.5 basic groups per 10^4 grams. Alderton et. al. (1945) found that on electrodialysis of lysozyme solutions the pH gradually increased to 9.5, and a partial precipitation of lysozyme resulted. On subsequent adjustment of the pH to 11.0 re-solution was apparent.

As has already been mentioned, Longsworth et. al. (1940) found on electrophoretic examination of diluted egg-white that the component G_1 maintained a positive charge over the entire range studied (pH 3.9 - 7.8). Alderton et. al. (1945), in studying the electrophoretic behavior of purified lysozyme, found good agreement with the mobilities

reported for G_1.

(c) Composition

Lysozyme has been characterized quite completely as regards amino acid composition by the work of two groups of investigators (Fromegeot and de Garilhe, 1949, 1950; Lewis et. al., 1950; Canfield 1963). Prior to amino acid analysis, Fromegeot and de Garilhe fractionated the amino acids of HCL hydrolyzates by chromatographic methods into four groups; namely, the basic, neutral aromatic, acidic and the neutral non-aromatic groups, while Lewis et. al. (1950) carried out microbiological analyses directly on the hydrolyzates.

The data from the two laboratories agree fairly well for most amino acids. Fromageot and de Garilhe (1950) did not originally find proline or methionine in their fractionated hydrolyzates, but later verified the data of Lewis et. al. (1950) who found both of these amino acids to be present. Later, the complete amino acid sequence was independently determined by Canfield (1963).

The most interesting biological property of lysozyme is its lytic activity against bacterial cell walls during which D-glucosamine, muramic acid and reducing groups are liberated. Because the structure of egg-white lysozyme is known, considerable attention has been directed towards an understanding not only of its enzymatic properties, but also of the physical relationship between the enzyme and

its substrate.

Much attention has been given to the action mechanism of lysozyme: early work pointed to the importance of the tertiary protein structure (the three-dimensional structure was determined by X-ray analysis by Blake et. al., 1965, 1967a, b), the disulphide bonds, the E-amino groups (from lysine) and the carboxyl groups. Current evidence supports the view that the disulphide bridges are important (Fraenkel-Conrat et. al., 1951; Goldberger and Epstein, 1963) but some of these can be reduced without affecting enzymic activity (Isemura et. al., 1961; Caputo and Zito, 1961).

It is interesting that Manwell (1967), concerned with "molecular palaeogenetics," claims that lysozyme is closely related to ribonuclease and may have evolved from a common protogene. The widespread distribution of lysozymes throughout the animal and vegetable kingdoms and in the bacteriophage suggests that it may well have evolved early in the formation of living systems.

In the hen, qualitative genetic differences in lysozyme have been found (Baker, 1968); it has been suggested that the locus has two co-dominant alleles. Quantitative differences are also inherited (Wilcox and Cole, 1957).

(d) Stability, Inactivation, and Denaturation

Meyer et. al. (1936) reported that lysozyme was very stable to heat and cold, but unstable to alkali. Solution

in 2% acetic acid lost no activity when held at 100°C for 45 minutes. Neutral solution similarly treated lost all activity, while the same result was achieved in 5 minutes at pH 9.0. Alderton et. al. (1946) found no loss in activity at pH 9.0 or below, over a period of three months at 23°C and only a 25% loss took place at pH 11.0. At pH 12.0, however, 60% loss took place in 7 days. The same investigators found that in HCL solution, pH 3.0, at a temperature of 96°C., 9, 21, and 40% of the activity was lost after 10, 25, and 50 minutes, respectively.

The isolated lysozyme was found to be resistant to enzymic hydrolysis by trypsin, papain, mild proteinase, and bacterial proteinase (Alderton et. al., 1945). Pepsin hydrolyzed the material at a slow, but measurable rate. After heating in acid solution, however, the material, while still active, was markedly more susceptible to hydrolysis by the enzyme; trypsin, for example, inactivating 66% of the material, whereas before heating and under the same conditions no measurable destruction had resulted. Heat may, therefore, alter the lysozyme molecule, sensitizing it to enzyme action without altering the biological activity (Fevold, 1951).

Ovomucin

Ovomucin contributes about 2% to the total protein of albumen (Baker, 1968, 1.5%; Feeney and Allison, 1969, 2.9%).

It is an insoluble, fibrous, acidic glycoprotein with high carbohydrate content of 18.6% (Donovan et. al., 1970). Feeney et. al. (1960) and Donovan et. al. (1970) reported a sialic acid content of between 2.5 and 4% but Odin (1951) gave values as high as 8%. The hexosamine content is between 7 and 12% (Odin, 1951; Brooks and Hale, 1961; Robinson and Monsey, 1964; Donovan et. al., 1970). Donovan et. al. (1970) also reported the presence of about 7% hexose and about 2 and 14% respectively of sulphur and total nitrogen.

Ovomucin is insoluble because it is a large molecule and is fibrous. Its electrophoretic characters have not been resolved, nor have estimates been made of its molecular weight or isoelectric pH. However, Sharp et. al. (1951) did resolve it into three fractions on moving-boundry electrophoresis.

Ovomucin's natural function in egg-white is not known, but it inhibits viral haemagglutination (Lanni et. al., 1949); despite numerous suggestions to the contrary, its sialic component is probably not involved in this activity. Other suggestions have ascribed to ovomucin the origin of the gel-like qualities of egg-white, particularly since it appears in greater concentration in the chalaziferous region and the thick white. Moreover, much appears to be present as a complex with lysozyme (Hawthorne, 1950; Feeney and Nagy, 1952; Cotterill and Winter, 1954; Sugihara et. al., 1955; Brooks and Hale, 1959, 1961; Oades and Brown, 1965).

Flavoprotein

Rhodes et. al. (1958) first reported riboflavin in egg-white as a flavoprotein complex. Rhodes et. al. (1959) showed that albumin contained about 0.8 to 1% (see Table 14) of equal amounts of flavoprotein and its apoprotein.

Subsequent work (see Baker, 1968; Feeney and Allison, 1969) verified these findings and showed that the apoprotein had a molecular weight of between about 32,000 and 36,000 and air isoelectric pH between 3.9 and 4.1. Its amino acid composition (Feeney and Allison, 1969) (see Table 15) differs only slightly from that reported by Farrel et. al. (1969). There appear to be no free sulphydryl groups, although the protein is highly crossed-linked by eight disulphide bridges (Farrel et. al., 1969). It contains 14% of carbohydrate derived from mahnose, galactose and glucosamine (Farrel et. al., 1969). The protein contains 0.7% or 0.8% phosphorus; there are two forms, one containing seven phosphate groups and the other eight (Rhodes et. al., 1959). On starch-gel and paper electrophoresis it moves ahead of ovalbumin components (Rhodes et. al., 1959; Baker and Manwell, 1962; Baker et. al., 1966).

Its most obvious characteristic is its ability to bind the vitamin riboflavin (in 1:1 molar ratio), hence all riboflavin in egg-white is held in an extremely stable complex (Winter et. al., 1967); this is unusual in that the flavin moiety is riboflavin and not the customary flavin

mononucleotide or flavinadeninedinucleotide. It has been suggested that the form found in egg-white is identical with the plasma flavoprotein complex (Ostrowski et. al., 1962).

Clearly a possible function for ovoflavoprotein is to transfer riboflavin to the embryo, since riboflavin injected into riboflavin-deficient eggs will allow hatching (Hawes and Buss, 1965). However, the generally accepted relationship between riboflavin deficiency, "clubbed down: and poor hatchability (Lepkovsky et. al., 1938; Coles and Cumber, 1955) is in some doubt, particularly since riboflavin-deficient eggs do not necessarily lead to chicks with "clubbed down" (Hawes and Buss, 1965).

Ovomacroglobulin

Ovomacroglobulin forms about 0.5% of egg-white. It was first seen by Lush (1961) on starch-gel electrophoretograms and was described in more detail by Feeney et. al. (1963a). It is the heaviest egg-white protein, with possible exception of ovomucin, having a molecular weight of around 800,000; its isoelectric pH is between 4.5 and 4.7 (see Table 14). Feeney and Allison (1969) state that it has no special noteworthy features as a protein (see Table 15), but little is known of its major characteristics. It is absent in the turkey and many genetic lines of Japanese quail.

Its main interest lies in that it is the only component

of egg-white with a wide spectrum of immunological cross-reactivity (Miller and Feeney, 1964, 1966); it is strongly immunogenic. Further information on this aspect is to be found in Feeney and Allison (1969).

Ovoglycoprotein

Little is known of this acidic glycoprotein. It was first noted by Ketterer (1962, 1965). It has a molecular weight of 24,400 and an isoelectric pH of 3.9 and contains about 13.6% of hexose, 13.8% glucosamine and 3.0% sialic acid. Feeney and Allison (1969) suggest that it might be identical with an acidic fraction isolated by Rhodes et. al. (1960), which contained approximately 4% sialic acid. Montreuil et. al. (1965) also found a similar protein.

It possibly forms the most advanced band on starch-gel and may be heterogeneous. It has no antihaemagglutination activity and inhibits neither trypsin nor chymotrypsin.

Ovoinhibitor

Matsushima (1958) first described a protein in chicken egg-white which inhibited bovine chymotrypsin (Rhodes et. al., 1960; Feeney et. al., 1963a). Further characterization (Rhodes et. al., 1960: Tomimatsu et. al., 1966) established that it would inhibit both trypsin and chymotrypsin in the ratio of two molecules of each proteinase to each molecule of protein; hence four molecules of enzyme could be inhibited

by each molecule of ovoinhibitor.

Ovoinhibitor occurs in albumen at concentrations between about 0.1% (Baker, 1968) and 1% (Feeney and Allison, 1969). The isoelectric pH is about 5.2 (see Table 14). It is composed of a single polypeptide chain (Davis et. al., 1969) of the amino acid composition given in Table 15. Ovoinhibitor is a glycoprotein containing about 3.5% hexose and 2.7% hexosamine, with little sialic acid (Rhodes et. al., 1960).

Ovoinhibitor migrates on starch-gel just ahead of the ovotransferrin bands (Tomimatsu et. al., 1966). It is not homogeneous and Tomimatsu et. al. (1966) distinguished three forms whilst Davis et. al. (1969) described five which differed only with respect to the carbohydrate moiety.

Avidin

This is a minor component of egg-white (0.05%). It binds biotin and was first described by Eakin et. al. (1940, 1941). Early physico-chemical studies gave a molecular weight of 48,000 to 66,000 and an isoelectric pH of 9.5; it was thought to consist of three peptide chains, each capable of binding one mole of biotin (Melamed and Green, 1963). Recent work suggests that it is composed of four chains and has a molecular weight nearer 70,000 (Green, 1964). Its amino acid composition has been determined (Table 15) (Melamed and Green, 1963).

Avidin may occur in three forms (Melamed and Green,

1963), two of which differ in composition whilst the third is complexed with another substance. Fraenkel-Conrat et. al. (1952) have previously described three forms composed of avidin plus nucleic acid, avidin-A formed from this complex and avidin complexed with an acidic glycoprotein. As it has not been possible unequivically to demonstrate avidin on starch-gels (Baker and Manwell, 1962; Lush, 1964), it has not been established whether the different forms represent genetical variants.

The importance of avidin lies in its capacity to bind three moles of biotin to about 64,000 g of protein as an extremely stable complex (Feeney and Allison, 1969) and so render biotin unavailable as a vitamin or coenzyme. In this way it can act as an antibacterial agent.

Minor Proteins

Several acidic proteins containing sulphydryl groups have been reported in egg-whites of different birds (Feeney et. al., 1960, 1966). The amounts present are extremely small, and little is known about their properties. Feeney et. al. (1966) and Baker and Manwell (1967) suggest that some cathodallymigrating proteins may be present.

Enzyme Inhibitors

The following were found to be enzyme inhibitors:

(I) Ovomucoid (see page 31)

(II) Ovoinhibitor (see page 42)

(III) Papain Inhibitor

(IV) Trypsin Inhibitor

Papain Inhibitor

Fossum and Whitaker (1968) have partially characterized a protein, comprising about 0.1% of egg-white, which inhibits both papain and ficin. Its molecular weight may be 12,700; apparently it is devoid of carbohydrate.

Trypsin Inhibitor

Kanamori and Kawabata (1964) identified trace amounts of an acidic protein thought to contain flavin; they suggested that it had antitryptic properties.

Enzymatic Proteins

The enzymatic proteins of egg-white are of the following:

(a) lysozyme (see page 34)

(b) glycosidases

(c) catalase activity

(d) peptidase

(e) esterases

Glycosidases

Lush and Conchie (1966), in hen's egg-white, discovered two enzymes, L-mannosidase and B-N-acetylglucosaminidase

(E.C. 3.2.1.30). The latter splits aryl-N-acetyl glucosaminides and more correctly it should be called B-2-acetamido-2-deoxy-D-glucosidase.

Catalase Activity

Lineweaver et. al. (1948) reported that egg-white had catalase activity as well as very slight esterase and peptidase activities. However, as pointed out by Baker (1968), care must be taken to distinguish between catalase activity (decomposition of H_2O_2) and the substance catalase itself.

On starch-gels, catalase activity seems to be associated with the ovalbumin and transferrin region in the fowl (Baker and Manwell, 1962; Corbin and Brush, 1966) and in other birds (Baker, 1965; Baker et. al., 1966; Baker and Manwell, 1967), but this activity may be artefactual (Baker and Manwell, 1962; Corbin and Brush, 1966).

True catalase has not been found in the fowl but Lush (1966) reported its presence in the quail; this was confirmed by Baker and Manwell (1967). Its migration on starch-gel is consistent with a molecular weight of at least 200,000. Further characterization has not been carried out. Catalase is a heam-protein, the function in quail egg albumen is not known. Dawson et. al. (1959) list H_2O_2 as an inhibitor of lysozyme, which suggests that albumen catalase may fill a protective role but Lush (1966) viewed it unlikely to have any important physiological function because it is absent

from chicken egg albumen.

Peptidases

Despite the original caution regarding peptidases in egg-white, Manwell et. al. (1967) reported that chicken egg-whites gives two zones on starch-gels which have been shown to be true peptidases.

Esterases

Egg-white of many birds shows esterase activity, but there is considerable inter- and intra-generic and specific variation (Baker and Manwell, 1962, 1967; Baker, 1965; Baker et. al., 1966). Moors and Stockx (1966, 1968) have reported the presence of alkaline and acid phosphomonoesterases and phosphodiesterases.

Japanese Quail Egg (Coturnix coturnix japonica Egg)

Interest in the quail as a domestic bird has been renewed and it is kept for meat and egg production and as a laboratory animal.

It is now possible to resolve Coturnix egg-white into at least twelve distinct proteins and the results of two-dimensional electrophoresis suggest that there are more (Baker and Manwell, 1967). The literature cited and information reported here are mainly of Baker and Manwell (1967), as the author cannot find any other material related to the

same subject.

Aging of Coturnix Egg-White

When whole Coturnix eggs or separated egg-whites have been stored at 0-4°C the electrophoretic resolution of proteins deteriorates noticeably. At higher temperature, the deterioration starts sooner and is more rapid. Ovalbumin is one of the soonest to deteriorate (Baker and Manwell, 1967).

Identification of Individual Proteins of Coturnix Egg-White

Figure 2 shows the composite diagram of coturnix egg-white proteins observed in several different buffers. The diagram has been expanded to include all the observed phenotypes. The trace proteins are only visible in pH 5.7 acetate gels under particularly favorable conditions.

Catalase

As mentioned before true catalatic activity is found only in Japanese quail and not in chicken. Baker and Manwell (1967) confirmed the report by Lush and Conchie (1966).

Esterase

Esterase is found in the Coturnix egg-white. The esterase does not stain well in the acid phosphate gels, in contrast to many other esterases (Baker and Manwell (1967).

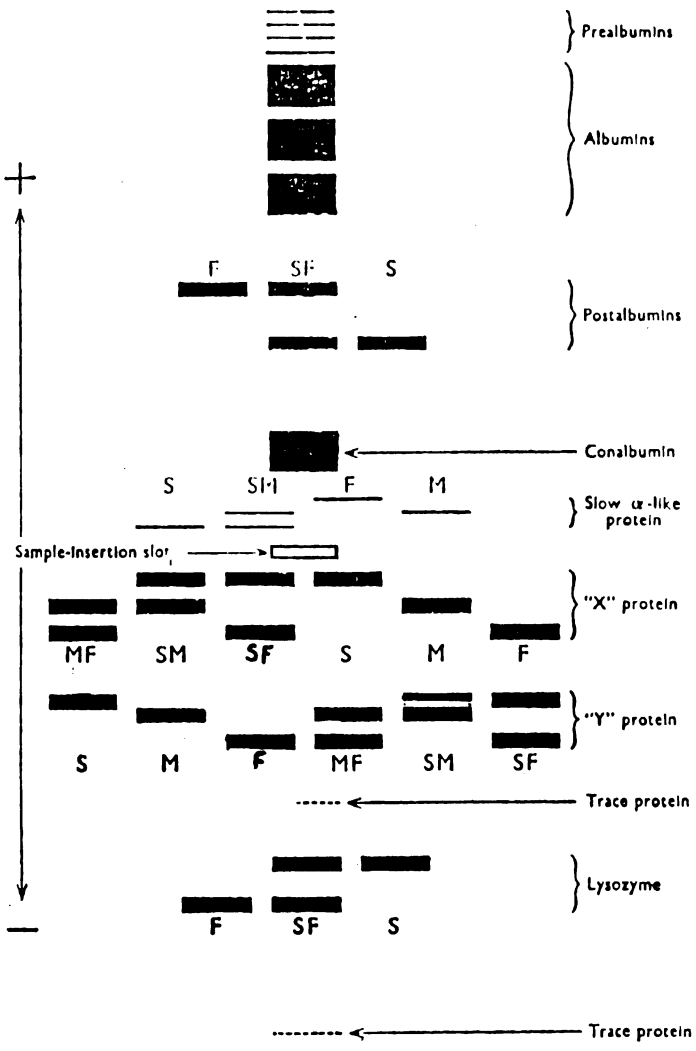

FIG. 2. Composite diagram of *Coturnix* egg-white proteins observed in several different buffers. The diagram has been expanded to include all the observed phenotypes. The trace proteins are only visible in pH 5·7 acetate gels under particularly favourable conditions.

Peptidase

Some of the peptidases located with napthyl conjugates are very active. Four zones of "peptidase" are observed after electrophoresis of Coturnix egg-white, using three substrates (Manwell et. al., 1967). The real nature and significance of these enzymes are unknown, but they appear to be widely distributed.

Lysozyme (G_1 Globulin; Muramidase)

Lysozyme is the most cathodal of the major proteins of coturnix egg-white. There are two types of coturnix lysozyme, "fast" and "slow." The genetic interpretation of these data is that the "fast" and "slow" only are homozygotes and the two-banded pattern of "fast" and "slow" represents the heterozygote. Preliminary studies on the activity of the three lysozyme phenotypes on bacterial cell wall substrate did not reveal any marked differences in kinetic properties.

Slow -α- Like Protein

This protein corresponds electrophoretically to a post-conalbumin slow -α- like protein in the domestic fowl and pheasant (Baker and Manwell, 1962; Baker et. al., 1966). It is probable that these slow -α- like proteins also correspond to "band 18" or ovomacroglobulin, a high molecular weight protein (Miller and Feeney, 1966).

X - Protein

This protein is called "X" temporarily because it has not yet been possible to find any clear similarity between it and any of the characterized chicken egg-white proteins.

Y - Protein (Ovomucoid)

The coturnix egg-white protein referred to as "Y" has some of the characteristic properties of ovomucoid. It can be obtained by methods used to purify ovomucoid from the egg-white of chicken (Lineweaver and Murray, 1947; Warner, 1954) and other species (Stevens and Feeney, 1963); "Y"-protein also has strong trypsin-inhibiting activity as reported by Feeney (1964). This report was supported and confirmed by Baker and Manwell (1967). From the information concerning the migration of chicken ovomucoid and ovoinhibitor in starch-gel (Tomimatsu et. al., 1966), Y-protein would seem more likely to be ovoinhibitor and postalbumin to be ovomucoid.

Postalbumin

Baker and Manwell (1967) found that postalbumin protein is also present in TCA-acetone-precipitated "ovomucoid." They reported that it was possible that a true ovomucoid or ovoinhibitor may be in this region.

Ovalbumin

Attempts to purify Coturnix ovalbumin by "salting out" with ammonium sulphate were unsuccessful (Baker and Manwell, 1967). Feeney et. al. (1966) also failed to crystallize ovalbumin by this method from the Adelic penguin egg-white. Individual variation in Coturnix ovalbumin was reported by Baker and Manwell (1962). Whether the variation represents actual molecular mutation in the ovalbumin, or in the enzymes involved in phosphorylation during ovalbumin synthesis in the oviduct, or in any enzyme capable of dephosphorylating the finished ovalbumin in egg-white is not known (Baker and Manwell, 1967).

Prealbumins

The prealbumins have not been studied extensively as the optimum conditions for their resolution are suboptimal for most of the other egg-white proteins. Four prealbumins can be resolved and some variation among individuals in the presence or absence of one band has been noted.

Other Interesting Observations

Ames (1966) used Japanese quail to test the effect of DDT on the hatching of osprey eggs. There were no differences in mortality and hatchability between control quail and the groups fed varying levels of DDE and DDD up to 50 ppm. This finding suggests that Coturnix are unsuitable for testing the

toxicity of pesticides. The results may give a false idea that certain compounds are relatively harmless.

It is also of interest that Ames (1966) DDE and DDD fed quail deposited large amounts of these substances in their eggs. In this connection it is worth noting that the Greeks and Romans were aware that Coturnix could cope with a diet including natural substances poisonous to man and for this reason, did not eat the birds (Zeuner, 1963).

The author had read in Chinese literature that in the olden day, Japanese quail eggs were served only to the Kings and that there were instances of therapeutic effects of quail eggs. For these reasons quail egg albumin was used in the observation later by the author.

Physiologic Availability of Nutrients in Eggs

The classic work of Atwater and Bryant (1899) concerned with the availability of proteins and fats of eggs revealed that approximately 97% of the proteins and 95% of the total fat were absorbed by healthy human subjects. Of many items tested, no other food was found to be superior in these respects. Research dealing with the utilization of amino acids indicates that the amino acids of eggs are readily available to the consumer.

Guthneck, et. al. (1953) observed that 98% of the lysine of dried whole egg was utilized to support weight gain in protein-depleted rats. Murlin et. al. (1938)

demonstrated the excellent performance of animals receiving egg proteins. On the other hand feeding experiments conducted by Boas (1927) and Parsons (1931) demonstrated that high levels of unheated egg-white produced a detrimental effect on test animals. Following numerous studies of the "toxic" effect of a substance occurring in egg-white, Parsons and Kelly (1933) concluded that the active material was proteinaceous in nature. The harmful effect disappeared when egg-white was heated. A crystalline material isolated from egg-white and capable of producing lesions similar to those described by Boas and Parsons was announced by Pennington et. al. (1942). This substance, named avidin was proved to combine with a vitamin (biotin) (see page 43), rendering the essential factor unavailable to test animals. Raw egg-white was, therefore, found to contain a physiologically active substance which combines with biotin to form a complex which the host cannot absorb.

Boyd et. al. (1966) fed raw egg-white powder to rats at a dose of 50 gm/Kg of diet/day, produced inhibition of growth, anorexia, diuresis, glycosuria, proteinuria, diarrhea, a decrease in weight of most organs except brain, gastrointestinal tract, salivary glands and kidneys and dehydration of several organs.

Peters (1967) produced a toxic syndrome by giving levels of 20%, 40%, 60%, 80%, or 100% of raw egg-white to young adult female albino rats. The toxic signs caused by

increasing intake of dried raw egg-white powder were: decreased food intake, weight loss, soft stool, diarrhea, glycosuria and death. Water intake and urine output rose with increasing raw egg-white powder in the diet. In all groups the urine was alkaline and the urinary output of protein increased. At autopsy there was a decrease in the absolute weight and in the water content of most body organs with increasing amounts of raw egg-white powder in the diet. The toxicity syndrome was not prevented by a biotin supplement, but was largely prevented by heat denaturation of the egg-white powder; 80% of denatured egg-white was well tolerated, as was 80% of casein in the diet. This indicated that the syndrome was due to the direct toxic effects of large amounts of dietary raw egg-white powder and not to biotin deficiency. The results of this study showed that inclusion of as little as 20% raw egg-white powder, but particularly of 80% or more, in the diet produced measurable signs of acute toxicity in adult female and young male rats. Bateman's (1916) original description of reduced food intake, weight loss, some development of tolerance after 7-10 days, soft stool and diarrhea was confirmed in the study by Peters (1967) and extended to include other clinical and pathological measurements. The results indicated that the toxicity syndrome originally reported by Bateman (1916) was due to biotin deficiency, and also to the direct toxicity of raw egg-white powder.

Embryological Functions of Egg-White

There is very little information on the function or importance of egg-white proteins in embryological development. Unproven but possible functions of the egg-white proteins during the development of the embryo are the following:

(1) A protective aqueous physical environment for the developing embryo on the surface of the egg yolk.

(2) A source of water and protein to the developing embryo. The embryo apparently "drinks" the egg-white during development.

(3) A source of certain particular constituents, such as the ovotransferrin, for transfer to the blood.

(4) A direct functional activity of a nutritional nature. One of these may be transport of calcium.

(5) An antimicrobial barrier and protection for the embryo.

(6) Buffer.

Direct evidence for any of these functions is apparently available. Studies have involved a removal of the egg-white during incubation of the embryo and its replacement by various substances. A preliminary interpretation of this data is that the egg-white may prove to be not particularly important physiologically but may be utilized for its nutritional value.

As early as 1890, Wurtz noted that various organisms, such as typhoid bacilli and pythogenic cocci, could not survive in egg albumen. Albumen, when left in an open, sterile dish,

may remain free of bacteria growth for as long as two months (Laschtschenko, 1909, quoted by Romanoff and Romanoff, 1949).

The egg's chemical defenses against bacteria are contained in the albumen. The ability of the albumen to protect the yolk from contamination by the microorganisms of the external environment is clearly shown in figure 3. Bacteria, after penetrating through the shell and membranes, multiply in the albumen for a short time and then rapidly decrease in number. Contamination of the yolk is delayed and comparatively light.

It has been recognized for many years that the failure of bacteria to grow in egg albumen is due chiefly to the presence there of a substance, or substances, possessing germicidal activity (Laschtschenko, 1909; Rettger and Sperry, 1912). The Romans reputedly recommended egg-white for the treatment of eye infections. The most interesting discovery was made by Fleming (1922) (see lysozyme). Since then many other egg-white proteins which show antimicrobial or anti-enzyme properties could be considered as potential microbial antagonists. These include lysozyme, ovoflavoprotein, avidin, ovotransferrin and the inhibitors of proteolytic enzymes, ovomucoid, and ovoinhibitor. Lysozyme has received the most notoriety and indeed is bacteriacidal to a limited number of species. Until fifteen years ago there was only a slow acquisition of data concerning the action of this enzyme (Thompson, 1940, 1941; Salton, 1957) but it was established

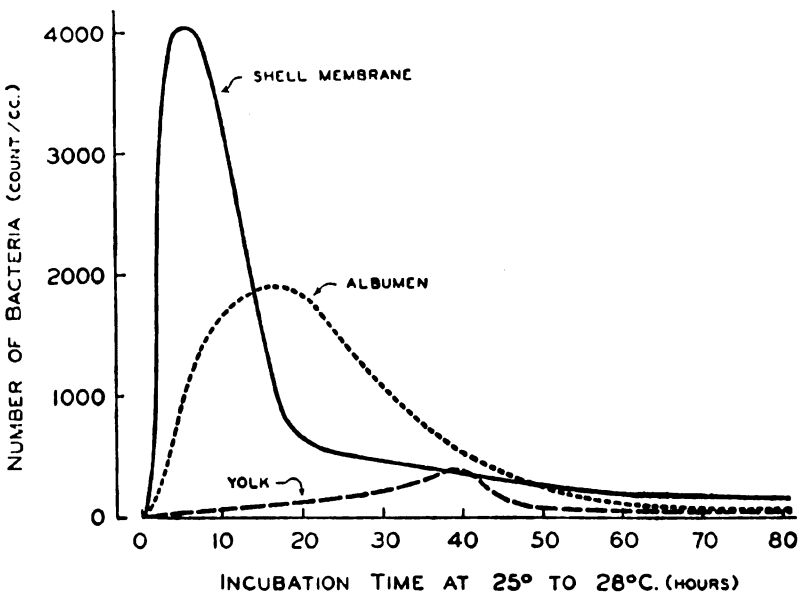

FIG. 35. The extent of contamination in shell membrane, albumen, and yolk observed during 80 hours' incubation of eggs smeared externally with a culture of *Pseudomonas aeruginosa*. (After Stuart and McNally, 1943.)

that under normal conditions gram-positive bacteria are on the whole more sensitive to lysozyme than are gram-negative organisms. The factor almost entirely overlooked, however, is the antibacterial activity of the high pH of egg-white during the first few days of incubation of the egg.

Ovotransferrin has been directly proven to be an important antimicrobial substance in a rather unique manner. When eggs are washed for commercial sales, there was a large difference in the rate of spoilage which could not be explained on the basis of sanitation and source of the eggs (Garibaldi and Bayne, 1962). Most people will not buy eggs if the shell has dirt and feces on them. The washing process, while removing the dirt, will, if not properly done aid in transporting microorganisms through the thousands of pores of the egg shell. After a long period of research it was shown that the reason for the difference in subsequent spoilage of the eggs was directly related to the iron content of the water used for washing. The higher the iron content, the more the subsequent spoilage and growth of microorganisms in the egg-white. The conclusion was that the small amounts of iron gaining entrance into the egg-white or in the pores of the shell was sufficient for microorganisms to initiate growth even in the presence of the high concentration of the iron chelating agent, ovotransferrin.

The biological properties of the proteins of the albumen are given in Table 16. These data have provided the

TABLE 16

THE BIOLOGICAL PROPERTIES OF COMPONENTS OF THE ALBUMEN OF THE CHICKEN'S EGG

Component	Action	Investigator
1. Lysozyme	(a) Lysis of cell walls of certain bacteria	Laschtschenko (1909)
	(b) Flocculation of bacterial cells	Fleming (1922)
	(c) Hydrolysis of B-1, 4-glycosidic	Freidberger and Hoder (1932) Berger and Weiser (1957)
2. Conalbumen (Ovotransferrin)	Chelation of iron, zinc and copper	Shade and Caroline (1944) Alderton, Ward and Fevold (1946)
3. Ovomucoid	Inhibition of trypsin	Balls and Swenson (1934) Lineweaver and Murray (1947)
4. Avidin	Combination with biotin	Eakin, Snell and Williams (1940) Woolley and Longsworth (1942) Baumgartner (1957)
5. Riboflavin	Chelation of Cations	Feeney and Nagy (1952)
6. Uncharacterized	(a) Inhibition of trypsin and chemotrypsin	Rhodes, Bennett and Feeney (1960)
	(b) Inhibition of fungal protease	Matsushima (1958)
	(c) Combination with riboflavin	Rhodes, Bennett and Feeney (1959)

TABLE 16 (Con't.)

Component	Action	Investigator
	(d) Combination with vitamin B_6	Evans, Butts and Davidson (1951)
	(e) Chelation of calcium	Abels (1936)

Source: Board, 1968.

basis for the currently accepted concept of the antimicrobial defense of the albumen, namely that it is a medium unsuitable for microbial growth (Board, 1968).

Contamination of the Egg

Research in the field of microbial deterioration of shell eggs still remains a problem to the poultry industry. The available literature found for marshalling evidence has been attempted in four publications only (Haines, 1939; Romanoff & Romanoff, 1949; Brooks and Taylor, 1955; Board, 1966).

In spite of the numerous microorganisms on the surface of the shell, bacteria are not usually found in the yolk in more than 10 percent of all fresh eggs or in albumen in more than 3 percent (Haines, 1939; Romanoff and Romanoff, 1949)

although higher percentages of contamination have been reported by some workers. Many workers have claimed that the shell is easily invaded following laying (Ferdinadov, 1944; Lorenz et. al., 1952; Graves & MacLaury, 1962) and that degree of contamination of the contents of fresh eggs is related directly to the porosity of the shell (Kraft et. al., 1958). When reviewing this facet of egg microbiology, Brooks and Taylor (1955) were forced to generalize that "roughly" 90% of newly laid eggs are free from microorganisms and the true value may be even higher.

The commonest contaminants of the contents of fresh eggs are micrococci which grow poorly, if at all, at the body temperature of the hen (Hadley and Caldwell, 1916; Haines, 1938; Miller and Crawford, 1953). Micrococci have been recovered also from ova taken from hens which had been killed and dissected in the laboratory (Harry, 1963). Harry (1963) favored the view that blood-borne organisms are primarily responsible for contamination of the ova. There is ample evidence (Rettger, 1913; May, 1924; Buxton and Gordon, 1947; Gordon and Tucker, 1965) that pathogens such as salmonella species pass from the alimentary canal via the blood to the ovaries, but there is no conclusive evidence of such migration by organisms capable of rotting eggs. Brooks and Taylor (1955) summarized in their observations that rot producing organisms are primarily of extragenital origin and that less than 1% of naturally clean eggs rot during prolonged storage.

When eggs were collected aseptically at the time of oviposition, Stuart and McNally (1943) recovered organisms from the shells of two eggs only. This suggests that the shell of a few eggs are contaminated when passing through the cloaca but that the main contamination occurs after laying. Fertile and infertile eggs ordinarily are contaminated to an approximately equal extent (Bushnell and Maurer, 1914; Hadly and Caldwell, 1916). The slightly lower incidence of contamination found in fertile eggs in one study (Rettger, 1913) is possibly without significance.

Gram-positive bacteria are numerically dominant on clean or slightly soiled shells whereas gram-negative ones can be dominant on badly soiled eggs. These contaminants are almost certainly derived from dust, soil, and faeces (Haines, 1939; Zagaevsky and Lutikova, 1944; Board et. al., 1964).

Board and Board (1968) characterized and identified the commonest contaminants as members of the genera Alcaligenes, Achromobacter, Pseudomonas, Serratia, Cloaca, Hafnia, Citrobacter, Proteus and Aeromonas.

Viruses such as Lymphoid Leukosis Virus (LLV), Infections Bronchitis Virus (IBV), Avian Encephalomyelitis Virus (AEV), Newcastle Disease Virus (NDV), and other viruses of an infected hen may transfer the infection to the egg in the oviduct at some stage between development of the ovum and completion of the egg in the oviduct or

infection can also be due to extragenital contamination by the virus.

The occurrence of egg transmission of LLV was firmly established by the work of Burmester (1962), Rubin et. al. (1961), and Rubin et. al. (1962). Infection by IBV is widespread in commercial flocks. Fabricant and Levine (1951) showed that IBV could be isolated from the eggs from infected hens up to 36 days after infection. Egg transmission of AEV is an important natural route of spread (Galnek et. al. (1960). Transmission occurs when the ovulating hen becomes infected and ceases when immunity develops. Egg transmission of NDV appears to be rare. DeLay (1947) and Hofstad (1949) isolated NDV from dead embryos and infertile eggs from infected stock, and DeLay also isolated the virus from hatched chicks. Hofstad found that egg transmission ceased as egg production recovered following outbreaks of the disease.

In addition to the disease-associated viruses mentioned above other viruses of little or no pathogenicity occur in the fowl and may be transmitted to the embryo. Note that most of the viruses are found in the egg yolk.

Mycoplasma gallisepticum is widespread in the field where it is responsible for respiratory infections of chickens and turkeys. In addition to spread by contact, egg transmission occurs (Van Roekel et. al., 1952; Fahey and Crawley, 1954). Yoder and Hofstad (1964) reported isolation of the organisms from ovary, oviduct and semen of infected birds.

Immunological Comparisons

Egg-whites and, in particular, chicken albumin have been used in many immunological studies. In 1938 Lansteiner et. al. showed the immunological relationships of several avian species. Landsteiner's experiments are now classical and used both ovalbumin and hemoglobulins of several species. More extensive data on egg-whites using three different proteins, ovalbumin, ovotransferrins, and lysozyme came from Deutsch's laboratory at Wisconsin in the early 1950's (Wetter et. al., 1953). Turkey, guinea hen, pheasant, duck, and goose whites were analyzed with antibodies to chicken proteins and were found to cross-react with each other. These investigators also obtained evidence indicating that a major portion of the antibody response (immunogenicity) of rabbit to chicken egg-white was directly against minor unidentified protein components. These minor components were present in other egg-whites examined and cross-reacted strongly.

Ovalbumins of different species have been compared immunologically by many workers (Kaminski, 1962; Jennings and Kaplan, 1962; Fothergill and Perrie, 1966). Wilson and co-workers (1968) studied the complement fixation by antiserum against chicken ovalbumin with a large number of different egg-whites in twenty-two taxonomic groups. This type of study has been extended to lysozyme by Arnheim and Wilson (1967).

Miller and Feeney (1964) have employed immunoelectrophoresis and immunodiffusion for comparative immunological studies. Antibodies were prepared in the rabbit against chicken, Japanese quail, duck, and cassowary egg-whites as well as purified chicken ovalbumin and ovotransferrin and cassowary ovotransferrin. They confirmed the observations of Wetter et. al. (1952) that a minor component was found to be strongly antigenic and showed extensive cross-reactions between all species examined. This component was identified as component 18 named ovomacroglobulin noted on starch-gel electrophoresis by Lush (1961) and Feeney et. al. (1963b). It was found widely distributed in the various flocks of chickens at the University of California at Davis. It was absent from many genetic lines of Japanese quail and not found in turkey egg-white selected to obtain a population sample (Feeney and Allison, 1969).

The physical and chemical properties of an immunologically cross-reacting macroglobulin in avian egg-whites were studied by Miller and Feeney (1966). They reported that macroglobulin has comparatively high antigenicity in rabbits and cows and shows extensive immunological cross-reactivity when the proteins prepared from the egg-whites of different species are tested against antibodies to chicken protein. Orr (1971) and Smith (1972) found high titers of reagins which were obtained in rats by initial immunization with egg albumen. Vanes and Harink (1973) reported that about 50

percent of guinea-pigs immunized with egg albumen possess strong antibody activity in an immunoglobulin class with antigenic properties different from those of the well-known guinea-pig immunoglobulins. This immunoglobulin is present in normal guinea-pig serum in very small amounts and its concentration rises during immunization with egg albumen. Immunization with bovine insulin or bovine γ-globulin does not stimulate increased synthesis of this immunoglobulin class.

Antibodies usually inhibit enzymes, though in some cases they cause activation (Pollock, 1964; Suzuki et. al. 1969). In addition, their efficiency of neutralization of enzymic activities varies depending on their specificities (Imanishi et. al., 1969). Since different parts of a protein molecule play different roles in maintaining the function and conformation of the protein, the most probable explanation for the diverse functions of antibodies is that they differ in antibody specificity (Fujio et. al., 1971). Now detailed information on antigenicity of lysozyme has been obtained from many laboratories (Arnon and Sela, 1969; Habeeb et. al., 1969; Bonavida et. al., 1969; Strosberg and Kanarek, 1970; Young and Leung, 1970; Maron et. al., 1971; Percht et. al., 1971). Many immunological studies have been carried out on hen egg-white lysozyme because its primary and tertiary structures have been fully characterized. Knowledge of this enzyme's antigenic determination has been derived from studies of the immunological behavior of peptide

fragments by many investigators mentioned above.

Data have become available for the complete amino acid sequence of human leukemia lysozyme (Canfield et. al., 1971). In addition, preliminary X-ray crystallographic data on this lysozyme at a 6 Å resolution have indicated that the three-dimensional structure of the two lysozymes (HEL and human leukemic lysozyme) appears to be the same (Blake and Swan, 1971).

Lysozyme is an enzyme known to be widely distributed in vertibrates. If the structures responsible for the enzymic activity of the enzyme from different sources are exactly the same, it is unlikely that it will be possible to make an antibody which is specific for the active center of enzyme from all these sources. However, Canfield et. al. (1971) compared the amino acid sequences of human lysozyme and hen egg lysozyme (HEL) and found that the amino acid moieties differed at a considerable number of points near the catalytic site. Therefore, it may be possible to make an antibody which is specific for the region close to the catalytic site of enzyme.

Hill and Sercarz (1974) studied the cellular and humoral antibody response in C57BL/6 mice to egg-white lysozyme (HEL) and six other immunochemically related lysozymes: bob-white (BEL), guinea hen (NEL), Japanese quail (JEL), pea-fowl (PEL), ringed-neck pheasant (REL) and turkey (TEL). Ninety percent of the animals are completely unresponsive to HEL, BEL, NEL,

and PEL, while the remaining 10% respond at a very low level and exhibit relatively restricted patterns by isoelectric focusing. All C57BL/6 mice are strong responders to JEL, and REL, and moderate responders to TEL.

Egg-White Protein and Poultry Breeding

The discovery of genetic polymorphism of egg-white raised hopes that it would provide information of use in breeding practice (Baker, 1960; Lerner and Donald, 1966). Egg-white protein polymorphism combines the advantages of discrete genetic characters and gene expression is the object of economic interest. Several of the larger poultry firms have typed their stocks for variants but the results have not been publicized. A measure of the success of egg-white polymorphism as a breeding tool is the fact that a number of poultry breeding firms have discontinued their typing; other firms only continue to type as a check on pedigree records (Baker, 1968). A check on the accuracy of pedigrees is a useful asset for which protein polymorphisms are idealy suited (Baker and Manwell, 1962, 1967).

Dried Egg-White

Dehydration is a successful way of preserving eggs. Research has played a major role in solving problems which involve stability, functional properties and quality of dried egg products. Dried eggs have the following advantages:

(a) They can be stored at low cost under dry storage or refrigeration with reduced space requirement.

(b) Transportation cost is low because water has been removed.

(c) They are easy and clean to use.

(d) They can be used in, and are necessary for, many new convenience foods.

Eggs can be divided into two basic categories when considering their drying characteristics:

(a) Egg-white products

(b) Whole egg and yolk products.

Egg-white products are virtually fat free, while whole egg and yolk products contain the highly emulsified lipids which are closely associated with the proteins and other components of the yolk.

Almost all dried-egg products are uncooked. For eggs to be useful, the native characteristics of the raw egg must be preserved (Bergquist, 1973). The density of egg products is not affected by dehydration. Since water is an integral part of the protein molecule, its removal may cause certain changes to occur in the properties of egg-white.

Egg-white is pre-treated in several different ways before drying. The primary purpose of pre-treatment is to remove the glucose. Acids are added to egg-white primarily for the purpose of pH adjustment. Various methods of treating egg-white before drying have been reported (Epstein,

1933; Balls and Swenson, 1936; Littlefield, 1938; Mulvany, 1941). Some of these methods involve the use of acids and/or enzymes.

Whipping aids are added to egg-white products to give them more uniformity and to compensate for any changes that might occur during processing and drying. The most common whipping aids used commercially and approved by FDA include sodium lauryl sulfate (Mink, 1939), triethyl citrate (Kothe, 1953), sodium desoxycholate (Kline and Singleton, 1959), and triacetin (Maturi et. al., 1960). These additives are used at about 0.1% base on egg-white solids and depending on type of additive. There are also other additives which have been reported to improve whipping properties, including acetyle methyl carbinol (Jensen and Hale, 1954), polyphosphates (Funucane and Mitchell, 1954), salts of capric acid (Silliker et. al., 1959), and calcium stearyl-2 lactylate (Gorman and Keith, 1960).

Injections of Egg Albumen to Animals

Very few articles relating to the subject of injecting animals with egg albumen can be found. Most of the animals used in the literature cited, were laboratory animals.

When animals are injected with egg albumen, large absorption droplets form in proximal tubule cells of the mammaliam kidney which contain mitochondrial material and ingested albumen (Oliver, 1948; Oliver et. al., 1954; Rhodin,

1954). The NADH-D activity of the mitichondria that form the bulk of the droplets is rapidly lost and the number of mitochondria decreases simultaneously with droplet formation (Pugh, 1972). The large lysozymes gradually disappear and the affected cells can renew their mitochondria within a few days. The normal pattern of mitochondria distribution is soon restored in proximal kidney tubules after damage caused by administration of egg albumen. This restoration which is facilitated by the ability of kidney cells to elaborate mitochondria from other cell organelles in addition to the division of pre-existing mitochondria was reported by Pugh (1972). The rats were injected intraperitoneally with 100 mg egg albumen in 1 ml of saline.

Coe (1971) found that when hamsters were inoculated with soluble hen egg albumen (HEA) synthesis of antibody to HEA was restricted to one of the FS immunoglobulins (FSy-globulin). This selective induction occurred regardless of the amount of the HEA-saline injection (.001-5.0 mg) or the route of inoculation. In contrast, HEA in Freund's adjuvants induced synthesis of anti-HEA in both the $7Sy_1$ and $7Sy_2$-globulins even when as little as 0.1 ug of HEA was used. Repeated inoculations of hamsters with HEA-saline increased or maintained $7Sy_1$ antibody but did not induce HEA synthesis in the $7Sy_2$-globulins.

IV. EXPERIMENTAL PROCEDURES

A. Growing Swine

General

A total of 66 pigs were used in three trials. The treated groups were injected subcutaneously with egg albumen weekly for four weeks and compared to control groups injected with .85% physiological saline solution. In Trial III, one group was fed 1% egg-white powder in the basal ration and compared to control groups not injected with physiological saline solution. The pigs were maintained at MSU Swine Research and Teaching Center in pens with slotted concrete floors and had free access to water and feed at all times. Feed and growth data were collected at weekly intervals. Trial I was started on September 29, 1973, and completed on October 29, 1973. Trial II was started on April 1, 1974, and ended on April 29, 1974. Trial III was started on August 5, 1974, and completed on September 9, 1974.

Trial I

Sixteen pigs were randomly allotted into two lots of eight each with sex and weight balanced. After lotting them, one barrow died from injuries sustained on the second day due to fighting. This lot served as control. The average

weight of the fifteen pigs was 37.27 Kg. (82 lbs). The eight pigs in the treated group were injected subcutaneously with the following volume of egg albumen:

week 1 - 15 ml;

week 2 - 20 ml;

week 3 - 25 ml; and

week 4 - 30 ml.

The control group was injected with the same volume of .85% physiological saline solution. There was no noticeable side effects due to introduction of foreign protein. The feed used in this trial was "MSU 16 Percent Ration" the composition of which is shown in Table 17.

TABLE 17

COMPOSITION OF RATION - TRIAL I

Ingredients	%
Corn	79.0
Soybean Meal (49%)	18.0
Dicalcium Phosphate	1.0
Limestone	1.0
Salt	.5
VTM Premix (MSU)[a]	.5
Total	100.0

[a]Composition of MSU vitamin and trace mineral premix is shown in Table 18.

Trial II

Heavier pigs were used in this trial. Twelve pigs with an average of 79.9 Kg (174 lbs) were randomly divided by weight into two equal groups. One pig in the control group was found to have one lame hind leg and was removed from the trial. The volume of egg albumen and saline solution were given the same as in Trial I. MSU 12 Percent Ration was fed free choice throughout the trial. The sex was disregarded in this trial.

TABLE 18

MSU VITAMIN AND TRACE MINERAL PREMIX

Ingredients	Amount in 4.55 Kg (10 lbs.) of premix
Vitamin A, million	3.0 I.U.
Vitamin D_2, million	0.6 I.U.
Vitamin E, thousand	10.0 I.U.
Riboflavin	3.0 gm
Nicotinic Acid	16.0 gm
D-pantothenic Acid	12.0 gm
Choline Chloride	100.0 gm
Vitamin B_{12}	18.0 mg
Zinc	68.0 gm
Manganese	34.0 gm

TABLE 18 (Con't.)

	Amount in 4.55 Kg (10 lbs.) of premix
Iodine	2.5 gm
Copper	9.0 gm
Iron	54.0 gm
Antioxidant (Ethoxyquin)	45.0 gm
Carrier (Ground Yellow Corn) to bring total to 4.55 Kg	

The compostiion of ration is shown in Table 19.

TABLE 19

COMPOSITION OF THE RATION IN TRIAL II

Ingredients	%
Corn	89.2
Soybean Meal (49)	8.0
Dicalcium Phosphate	1.0
Limestone	.8
Salt	.5
MSU VTM Premix (see Table 18)	.5
Total	100.0

Trial III

Forty pigs with an average weight of 68.3 Kg (150.25 lbs.) were randomly allotted into four equal lots with weight balanced. They were treated as outlined in Table 20.

TABLE 20

OUTLINE OF EXPERIMENT IN TRIAL III

Lot No.	Sex	No. of Pigs	Treatments
1	6F, 4M	10	Basal feed 10% protein
2	3F, 7M	10	Basal feed (10% protein) plus 1% egg-white powder
3	6F, 4M	10	Basal feed (10% protein) plus egg albumen injection weekly
4	4F, 6M	10	Finisher feed 13%

In this trial only Lot No. 3 pigs were injected with egg albumen subcutaneously. Data on weekly feed consumption and weight gain were collected as in the other two trials. The levels of albumen used in this trial were much higher

than in the first two trials. Injections of weekly dosages of 40 ml, 50 ml, 55 ml, and 55 ml, respectively, were made. On the third week of injection it was found that too high a dosage of egg albumen injected subcutaneously behind the ear caused some stress and irritation at the site of the injection, therefore, the dosages for the next two weeks were kept at 55 ml per week. The composition of the rations used is shown in Table 21.

At the end of the fifth week, blood samples from five pigs of each lot were taken. The blood samples were obtained from the anterior vena cava of all animals by the technique described by Carle and Dewhirst (1942). Blood samples were taken again from the same five pigs in Lot 3, 24 hours after the injection of egg albumen. Fourteen ml of blood was obtained from each pig. An additional 2 ml of blood was placed in a heparinized vial to be used for hematocrit and hemoglobin determinations. All tubes for containing the blood samples were tightly corked except when they were being sampled. Serum samples were "rimmed" in the tube before putting them in the centrifuge. Separation of serum from cells was accomplished with the use of an international centrifuge, size 2, Model V, at 2000 x g for 25 minutes. Serum was removed, placed in vials and chilled

TABLE 21

COMPOSITION OF RATIONS IN TRIAL III

Ingredients	Basal 10% (Kg) (%)	Basal 10% + Egg-White (Kg) (%)	Finisher Ration 13% (%)
Egg-White Powder	0	1.0	0
Corn #2	91.5	90.5	85.0
Soybean Meal (49%)	5.0	5.0	11.5
Dicalcium Phosphate	1.0	1.0	1.0
Limestone	1.0	1.0	1.0
Salt	.5	.5	.5
MSU VTM Premix	.5	.5	.5
VIT E-Se Premix	.5	.5	.5
Total	100	100	100

in a cold room at 4°C for determination of ammonia N, urea N, and agar-gel electrophoresis. Later the serum was frozen for use in total serum protein determination. Determinations of hematocrit and hemoglobin were made within 2 to 3 hours after the samples were collected.

Hematocrit values were determined by the procedure outlined by McGovern et. al. (1955). Hemoglobin was determined by the cyanmethemoglobin method described by Crosby et. al. (1954). Conway's (1957) micro diffusion technique was used for serum urea N and serum ammonia N. Total serum protein was determined according to the Lowry's method modified and described by Waddel (1956). The serum protein fractions were separated on a spinco, Model R, agar-gel electrophoresis system. The technique used by Cawley and Eberhardt (1962) was followed very closely. The relative intensities of the separated proteins were determined by scanning with a Spinco Model RB Analytrol equipped with two 500 millimicron filters and a B-5 cam.

The rations in Trial III were analyzed for their energy values by using oxygen bomb calorimetry. The Kjeldahl technique was used to determine the nitrogen content of the feed. Table 22 shows the protein and energy values.

TABLE 22

PROTEIN AND ENERGY ANALYSIS OF FEED

		Energy	Protein
Lot 1	Basal Ration	3890.0 cal/g	10.8%
	Basal Ration	3916.9 cal/g	11.3%
Lot 2	Basal + 1% Egg-White	3973.0 cal/g	11.9%
	Basal + 1% Egg-White	3913.6 cal/g	12.0%
Lot 3	Basal Ration	3885.1 cal/g	12.3%
	Basal Ration	3872.9 cal/g	12.4%
Lot 4	Finisher Ration	3860.4 cal/g	12.6%
	Finisher Ration	3889.4 cal/g	13.1%

Carcass evaluation was also made and it was from five pigs of each lot. The results are shown under "Results and Discussion." The average weight of pigs at the time of slaughter was 91.76 kilograms.

B. Chickens

General

Three trials were conducted and each trial was composed of different types of chickens and at a different time and place. Trial I was started on May 5, 1973, and completed on May 29, 1973, with Barred Plymouth Rock. Trial II was started on July 27, 1973, and completed on August 24, 1973, with SCWL. Barred Plymouth Rocks were used in the same trial which was started on July 30, 1973, and completed on August 27, 1973. Trial III was started on October 29, 1974, and completed on April 2, 1975.

Trial I

Twelve Barred Plymouth Rock females, 7 weeks old, were used in this trial. They were divided into two lots of six birds each. One was used as control and the others were treated with egg albumen. Three ml of chicken egg albumen were injected subcutaneously at weekly intervals for 4 weeks and the growth data were collected.

These birds were kept in battery cages at one bird per cage in the cage room in the basement of Anthony Hall. Commercial grower mash was used.

Trial II

Forty SCWL chicks, three-weeks old, and 40 Barred Plymouth Rock chicks, three-weeks old, were used in this trial. They were sexed and then divided into eight lots as outlined in Table 23.

TABLE 23

OUTLINE OF AN EXPERIMENT FOR TRIAL II

Type of Chicks	Control	Treated
SCWL ♂	10	10
SCWL ♀	10	10
Barred Plymouth Rock ♂	9	9
Barred Plymouth Rock ♀	11	11
Total	40	40

All the chicks were fed with regular starter ration and were weighed at weekly intervals. The treated group was injected subcutaneously with chicken egg albumen at ½ ml, 1 ml, 1½ ml, and 2 ml each week, respectively, for

four weeks. Growth data were collected weekly. The birds were kept in a brooder battery in the cage room in the basement of Anthony Hall. Feed consumption data were not taken.

Trial III

Initially, 80 pullets were housed in House No. 5, pen A at the MSU Poultry Science Research and Teaching Center. Half of the pullets were hatched on April 23, 1974, and the other half on May 7, 1974. The birds were individually kept in battery cages. The birds were observed for two weeks and then they were allotted into lots A, B, C, and D with ten birds per lot. The age of the chicks in each group was equally balanced, i.e., five pullets hatched on April 23, 1974, and five pullets hatched on May 7, 1974, were put in each lot. Selection was also made so that the egg production of the four lots was about the same based on egg production obtained during the observation period. The rest of the birds were assigned to lots in E, F, G, and H. They were without age balance and were used for observation.

The outline of the experiment is shown in Table 24.

TABLE 24

OUTLINE OF EXPERIMENT IN TRIAL III (a)

Lots	Number of Birds	Treatments
A	10	Control, no treatments
B	10	Injected 4 ml chicken albumen
C	10	Injected 4 ml quail albumen
D	10	Injected 4 ml Ringer's solution

The treatments were started on November 13, 1975. The birds were injected with 4 ml of chicken albumen, quail albumen, or Ringer's solution each week for five weeks. Individual production data were collected during the period of treatment.

On February 18, 1975, lots E, F, G, and H were treated as outlined in Table 25, because lot C hens treated with quail albumen were showing some positive results.

After one week pre-treatment observation the birds were injected subcutaneously with 4 ml of quail egg albumen per week for five weeks. Individual egg production data were collected.

TABLE 25

OUTLINE OF REPLICATIONS OF QUAIL ALBUMEN TREATMENTS (Trial IIIb)

Lots	Number of Birds	Treatments
E	9	Injected 4 ml quail albumen
F	10	Control, no treatment
G	9	Injected 4 ml quail albumen
H	10	Control, no treatment

V. RESULTS AND DISCUSSION

A. Growing Swine

Trial I

A summary of the data obtained in this trial is presented in Table 26 and Appendix Tables 40 and 41. Pigs in each treatment were group-fed; therefore, a statistical analysis could not be made on feed consumption and feed efficiency data. The rate of growth was approximately the same for the treated and control groups as can be seen in Table 26.

As mentioned in the introduction, the author had made some observations in Malaysia and there was a significant increase in growth rate of pigs injected with egg albumen as compared to that of controls. In comparing this trial with the trial in Malaysia it could possibly have been due to any or all of the following:

	MSU	Malaysia
Breed:		
1.	Undoubtedly the pigs were of a superior breed	Poor breed
Age:		
2.	Used younger pigs (2½ months)	Used older pigs (5½ months)

TABLE 26

SUMMARY OF DATA IN TRIAL I

	Control	Treated
Number of Pigs	7	8
Average Initial Weight (Kg)[a]	37.41	37.22
Average final weight (Kg)[b]	58.18	57.33
Average Daily Gain (Kg)	0.74	0.72
Average Daily Feed (Kg)	1.78	1.74
Feed/Gain (Kg)	2.40	2.42
Feed Cost/Kg - Gain (12.1¢/Kg)	29.04	29.28
Gain in Weight/Pig (Kg)	20.77	20.11
Feed Cost During Period	$ 6.03	$ 5.89
Extra Labor Charge for Treatment	-	1.00
Cost of Feed and Treatment	$ 6.03	$ 6.89

[a]Standard error for control 0.96 and treated 0.89.

[b]Standard error for control 1.32 and treated 1.29.

	MSU	Malaysia
Diet:		
3.	High quality balanced diet	Poor quality diet
4.	Dry and free choice feed	Slop-feeding twice a day
Housing:		
5.	Controlled environmental housing	Pigs on concrete floor with low walls and a roof
Treatment:		
6.	Without bathing	Bathing the pigs twice a day to keep them cool

From this comparison it is obvious that an important factor has been overlooked—the age of pigs. The author recalled that he had tried the injection of egg albumen on younger pigs in Malaysia but with no response. A second trial was proposed and was approved.

Trial II

In Table 27 and Appendix Tables 42 and 43 are shown a summary of the data obtained in Trial II. No statistical analysis could be made on feed consumption and feed efficiency data because pigs in each treatment were group fed. Weight gain between the two groups was about the same. There was a fairly large difference in the feed efficiency which, however, could not be tested statistically. The treated group required only 3.3 Kg of feed per kilogram of body weight gain, whereas the control group required 4.1 Kg of feed. There was

TABLE 27

SUMMARY OF DATA IN TRIAL II

	Control	Treated
Number of Pigs	5	6
Average Initial Weight (Kg)[a]	79.46	79.09
Average Final Weight (Kg)[b]	98.64	98.18
Average Daily Gain (Kg)	0.69	0.69
Average Daily Feed	2.81	2.26
Feed/Gain (Kg)	4.10	3.30
Feed Cost/Kg Gain (11.66¢/Kg)	47.81	38.48
Gain in Weight/Pig	19.18	19.09
Feed Cost/Pig During Period	$ 9.17	$ 7.35
Extra Labor Charge for Treatment/Pig	-	1.00
Cost of Feed and Treatment/Pig	$ 9.17	$ 8.35

[a] S.E. for control = 2.69
treated = 2.32

[b] S.E. for control = 3.12
treated = 2.70

considerable saving of feed during the period of observation. Even though extra labor was charged for handling and treatment of pigs, a savings of 82 cents could be realized on the cost of feeding each pig (see Table 27).

The author's hypothesis is that pigs eat more than the body can absorb when feed is given free choice and the excess food that is not digested is excreted and wasted. Egg albumen depresses the appetite and thus controls the food intake. MSU pigs had reached their fullest potential for weight gain and egg albumen treatment did not stimulate any higher rate of growth. Malaysian pigs were given poorer types of feed and rate of growthwas below its own potential, thus egg albumen helped to stimulate growth. The author did not keep records on feed intake in Malaysia because the treated and the control pigs were kept in the same pen.

Carcass evaluation was also taken but it was found that there was no significant difference in the dressing-out percentage or the percent of loin between the two lots.

Trial III

Table 28 and Appendix Tables 44, 45, 46 and 47 show the summary of performance data of Trial III. A higher level of egg albumen injection (40 ml and above) was found to have some harmful effects though not very obvious. Two pigs were found to have soft stools and one was vomiting during the time of weighing. Two pigs developed abscesses at the sites

of injection. This may be due to injecting the high dosage of egg albumen at one time on the same spot subcutaneously or due to contamination which causes the abscesses. The abscesses were healed within a week but during that period there was no noticeable gain in weight. Although Lot No. 1 which was on basal diet showed a better gain in weight over Lot No. 3 which was treated with egg albumen injection, statistically there was no significant difference. In comparing Lot No. 3 with Lot No. 4 which was fed with 13% finisher ration, gain in weight was significantly ($P < 0.01$) greater in Lot No. 4. Also, in Lot No. 4 body weight gain was significantly ($P < 0.05$) greater than Lot No. 1, Since Lot No. 4 was the positive control, this result was expected.

The failure to improve weight and feed efficiency by injecting might be due to the harmful effect of too high a dosage. Lot No. 4 had the best utilization of feed gain among the four lots. Lot No. 1 was the poorest feed converter, Lots No. 2 and No. 3 had the same feed efficiency.

The poor performance of pigs injected with egg albumen might be due to some low level of toxicity such as that described by Harper et. al. (1955), Boyd et. al. (1966), Peters (1967), and Pugh (1972). The injected pigs had depressed appetites as can be seen in Table 28. The average daily feed intake was the lowest of all in Lot 3. Vomiting and loose stools may also indicate some toxicity.

TABLE 28

SUMMARY OF PERFORMANCE DATA IN TRIAL III

Lots	1	2	3	4
Treatments	Basal Feed 10%	Basal Feed + 1% egg white	Basal Feed + egg albumen injection	Finisher Feed 13%
Number of Pigs	10	10	10	10
Average initial weight (Kg)	68.14±	69.32±	68.23±	67.59±
Average final weight (Kg)	86.45±	88.41±	84.18±	91.68±
Average daily gain (Kg)	0.53*	0.59	0.51**	0.67
Average daily feed (Kg)	2.34	2.45	2.12	2.44
Feed/gain (Kg)	2.00	1.89	1.89	1.66
Feed cost/Kg gain (¢)	27.8	33.26	26.27	23.74
Gain in weight/pig (Kg)	18.27	20.32	17.45	24.09

*Significant difference ($P < 0.05$) as compared to lot 4.

**Highly significant difference ($P < 0.01$) as compared to lot 4.

The average values for hematocrit, hemoglobin, serum protein, urea N, ammonia N, electrophoretic pattern of growing pigs are shown in Table 29.

Analysis of hematocrit and hemoglobin data indicated no significant differences among treatments. Total serum protein was not significantly different between lots. There was a slight increase of .20 gm/100 ml of total serum protein level in the pigs 24 hours after injection of egg albumen. The total serum protein level for pigs in Lot 1 and Lot 3, seven days after albumen injection, was the same (7.41 gm/100 ml). This could also indicate that the effect of egg albumen was nil on the 7th day. Lot 4 (13% finisher) had the highest total serum protein level (7.87 gm/100 ml) and Lot 2 (Basal + 1% egg-white was second (7.60 gm/100 ml).

Duncan's multiple-range test was used and no statistically significant difference was found between treatments in the plasma ammonia N, plasma urea N, and in the electrophoretic patterns of the plasma proteins.

Table 30 shows a summary of the carcass evaluation. Although there is no statistically significant difference among the groups it is interesting to note that Lot 2 and Lot 3, both of which had been treated with egg-white, were 1.5% higher than Lot 1 in dressing-out percent and 1.1-1.6% higher in the percent of ham and loin. In comparing Lot 4 and Lot 1, Lot 4 with 13% finisher ration, was 0.6% higher in the dressing-out percent and 1.2% higher in the percent of ham and loin.

TABLE 29

HEMATOCRIT, HEMOGLOBIN, SERUM PROTEIN, UREA N, AMMONIA N, ELECTROPHORETIC PATTERN OF PIGS, TRIAL III

	Basal 10%	.1% Egg-White Powder & Basal	Injected and Basal [a]	[b]	Finisher 13%
Hematocrit, %	34.58	36.13	35.23	34.7	38.38
Hemoglobin gm/100 ml	11.20	11.26	10.59	11.10	11.53
Total Serum Protein gm/100 ml	7.41	7.60	7.41	7.61	7.87
Ammonia N mg/100 ml	1.93	2.91	3.58	1.23	4.17
Urea N mg/100 ml	13.09	12.14	9.59	11.31	13.34
Albumen %	25.01	26.85	25.28	25.25	26.85
α-Globulin %	33.67	31.76	31.62	29.83	32.99
B-Globulin %	11.76	12.78	11.33	15.45	12.89
γ-Globulin %	29.56	28.61	29.73	29.46	27.27

[a]Blood samples taken one week after egg albumen injection.

[b]Blood samples taken 24 hours after egg albumen injection.

TABLE 30

CARCASS EVALUATION, TRIAL III

	Average Body Weight (Kg)	Dressing %	Ham & Loin %
Lot 1	90.17	70.90	37.56
Lot 2	93.95	72.49	38.62
Lot 3	87.48	72.42	39.11
Lot 4	95.44	71.52	38.82

B. Chickens

Trial I

Although the results in Trial I showed that the body weight gain in the treated group was more than that in the control group statistically the difference was not significant. (Table 31 and Appendix Table 48). The average body weight gain for the treated group was 503 gm and for the control group it was 447 gm. The samples were not large enough to show any statistical difference in weight gain.

TABLE 31

RESULTS OF TRIAL I, BARRED PLYMOUTH ROCK, FEMALE

Bird No.	Initial Weight (gm)	Final Weight (gm)	Weight Gain
Control Group			
13	970	1,500	530
14	846	1,139	293
15	1019	1,409	390
16	994	1,417	423
17	918	1,405	487
18	1048	1,607	559
Total	5795	8,477	2,682
Mean	965.8±	1,412.8±	447±
Treated Group			
31	916	1,386	470
32	721	1,210	489
33	981	1,459	478
34	905	1,375	470
35	913	1,476	563
36	1014	1,562	548
Total	5450	8,468	3018
Mean	908.3±	1,411.3±	503±

Trial II

All the birds survived during the period of observation. It is interesting to note that the male birds were not affected by the albumen treatment. SCWL male control and treated groups had the same weight gain (see Table 32 and Appendix Table 49), and for Barred Plymouth Rock males the treated group gained less than the control (see Table 34 and Appendix Table 50). The female birds seemed to have some response tochicken albumen treatment. The improved body weight gain of the treated female birds were not significant from control (see Table 33 and Appendix Table 51; Table 35 and Appendix Table 52). A comparison between the same sex which is shown in Table 36, indicated that rate of growth of male birds appeared to be slightly depressed by chicken egg albumen injection. The treated male birds gained less than control male birds. In female birds there was an increase in body weight gain in the treated birds as compared to the control. A summary for all the 80 birds showed that there was no significant difference in rate of growth between the control and treated chickens (see Table 37).

TABLE 31

RESULTS OF TRIAL I, BARRED PLYMOUTH ROCK, FEMALE

Bird No.	Initial Weight (gm)	Final Weight (gm)	Weight Gain
Control Group			
13	970	1,500	530
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31	916	1,386	470
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33	981	1,459	478
34	905	1,375	470
35	913	1,476	563
36	1014	1,562	548
Total	5450	8,468	3018
Mean	908.3±	1,411.3±	503±

TABLE 32

RESULTS OF TRIAL II (a), SCWL MALES

Control Group		
Initial Weight (gm)	Final Weight (gm)	Weight Gain (gm)
204	709	498
195	573	378
190	681	491
194	642	448
158	537	379
219	697	478
218	700	482
197	722	525
173	558	385
201	636	435
Total 1949	6448	4499
Mean 194.9±	644.8±	449.9±

Treated Group		
203	759	556
195	526	331
186	657	471
199	687	488
165	582	417
219	684	465
207	681	474
204	668	464
184	607	423
161	571	410
Total 1923	6422	4499
Mean 192.3±	642.2±	449.9±

TABLE 33

RESULTS OF TRIAL II (b), SCWL FEMALES

Initial Weight (gm)	Final Weight (gm)	Weight Gain (gm)
Control Group		
182	529	347
207	566	359
185	547	362
161	487	326
123	418	295
204	537	333
170	515	345
160	490	330
188	539	351
197	540	343
Total 1777	5168	3391
Mean 177.7±	516.8±	339.1±
Treated Group		
180	575	395
211	606	395
146	463	317
204	584	380
173	521	348
174	481	307
159	520	361
196	581	385
199	632	433
196	545	349
Total 1838	5508	3670
Mean 183.8±	550.8±	367.0±

TABLE 34

RESULTS OF TRIAL II (c), BARRED PLYMOUTH ROCK MALES

Control Group		
Initial Weight (gm)	Final Weight (gm)	Weight Gain (gm)
313	964	651
312	886	574
343	964	621
324	1028	704
303	938	635
327	995	668
291	803	512
348	1039	691
295	890	595
Total 2856	8507	5651
Mean 317.3±	945.2±	627.9±

Treated Group		
315	690	375
312	961	649
317	865	548
344	836	492
325	1072	747
336	960	624
281	877	596
368	1040	672
285	831	546
Total 2883	8132	5249
Mean 320.3±	903.6±	583.2±

TABLE 35

RESULTS OF TRIAL II (d), BARRED PLYMOUTH ROCK FEMALES

Initial Weight (gm)	Final Weight (gm)	Weight Gain (gm)
Control Group		
318	819	501
308	757	449
285	779	494
296	721	425
252	741	462
255	700	445
304	783	479
269	721	452
242	718	476
282	651	369
288	763	475
Total 3099	8126	5027
Mean 281.7±	738.7±	457±
Treated Group		
303	866	563
308	830	522
297	741	444
252	685	433
255	719	464
304	812	508
298	764	466
272	681	409
249	745	496
304	826	522
286	755	469
Total 3128	8424	5296
Mean 284.4±	765.8±	481.5±

TABLE 36

SUMMARY OF RESULTS OF TRIAL II BY COMPARING THE GAIN IN WEIGHT OF THE MALE AND FEMALE SEPARATELY

	Control (gm)	Treated (gm)	Difference (gm)
Male			
SCWL	449.9±	449.9±	0
Barred Plymouth Rock	627.9±	582.2±	45.7±
Total	1077.8	1032.1	45.7±
Mean	538.9±	516.1±	22.9±[a]
Female			
SCWL	339.1±	367.0±	27.9±
Plymouth Rock Rock	457.0±	481.5±	24.5±
Total	796.1	848.5±	52.4±
Mean	398.1±	424.3±	26.2±[b]

[a]Control birds gain in weight more than treated in male.

[b]Treated birds gain in weight more than control in female.

TABLE 37

SUMMARY OF RESULTS OF TRIAL II WITH MALE AND FEMALE COMBINED

	Control Group
	Weight Gain (gm)
SCWL ♂	449.9
SCWL ♀	339.1
Barred Plymouth Rock ♂	627.9
Barred Plymouth Rock ♀	457.0
Total	1873.9
Mean	468.5±

	Treated Group
SCWL ♂	449.9
SCWL ♀	367.0
Barred Plymouth Rock ♂	583.2
Barred Plymouth Rock ♀	481.5
Total	1881.6
Mean	470.4±

Trial III

During the five weeks of treatment, no significant difference was found in the egg production data comparing treated to control groups (Figure 4). Three weeks after termination of treatments, egg production in Lot C, which was treated with quail egg albumen remained higher than those of the other groups until the data were no longer collected (9 weeks), (see Figure 4). Groups treated with egg albumen followed second but only for a three week period, and then dropped to about control level. This possibly indicated that quail egg albumen was biochemically more active than chicken egg albumen. Out of 107 days of egg production, Lot C produced 906 eggs, Lat A, control group, produced 803 eggs, Lot B, treated with chicken egg albumen, produced 797 eggs and Lot D, treated with Ringer's solution, produced 779 eggs (see Table 38 and Appendix Table 53). Statistical analysis showed a highly significant difference in egg production in favor of the hens injected with quail egg albumen (P 0.01). The average egg weight during the last six weeks is also shown in Table 38.

Chronic respiratory disease symptoms were observed in the poultry house where the research birds were kept on the eighteenth week of the experiment. As shown in Figure 4, egg production in all other lots were dropping on the eighteenth week of observation while Lot C (QEA) had an increase in egg production to 84.3%. On the nineteenth week egg

TABLE 38

SUMMARY OF EGG PRODUCTION DATA, TRIAL III

Lots	Days of Production	Total No. of Eggs	Average Egg Weight (gm)[b]	% of Lay	
				Hen-House	Hen-Day
A (CONT)	107	803	59.6	75.1	75.1
B (CEA)	107	797	60.6	74.5	74.5
C (QEA)	107	906	57.4	84.7	84.7*
D (RS)[a]	107	779	58.6	72.8	78.2

Control - (CONT); Chicken Egg Albumen - (CEA); Quail Egg Albumen - (QEA); Ringer's solution - (RS).

[a]Two hens died, one on the 12th week and one on the 13th week of the experiment.

[b]Last 42 days of experiment.

*Highly significant difference (P 0.01).

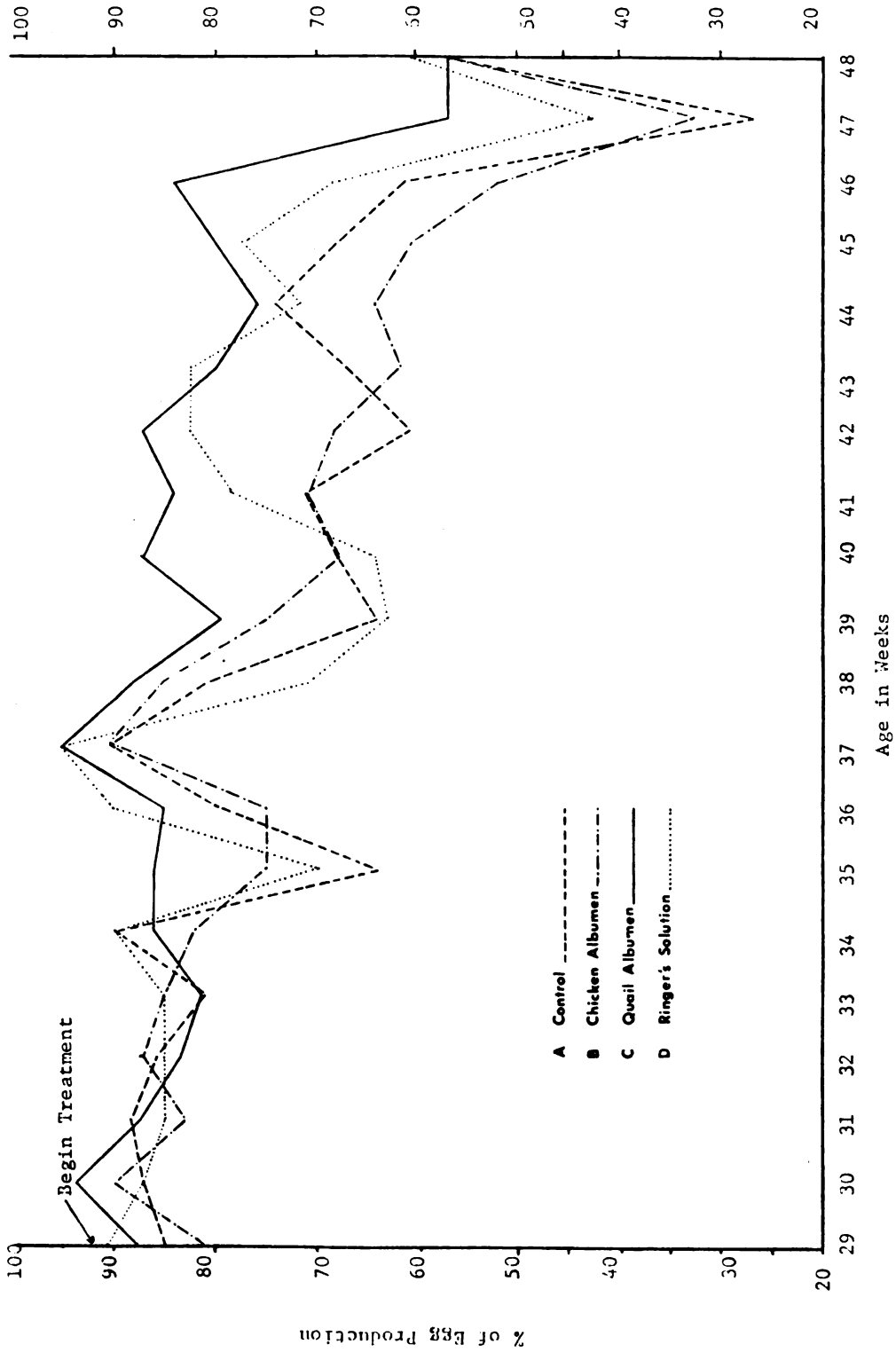

Fig.4: A Summary of Hen Day Egg Production

production dropped drastically for all the hens but Lot C (QEA) still maintained higher egg production than the other lots with 57.1%. This period might be the peak of CRD outbreak. During the next week (20th week) egg production for other lots began to show improvement over the week before while Lot C (QEA) still maintained its egg production as shown in Table 39. The hen-day production record of Lot D (RS) was the best of all, 60.7% during this period. Egg production did not drop during the initial stage of CRD outbreak for Lot C (QEA) and did not drop as low as others during the peak of CRD outbreak indicated that there might be an unknown factor or factors in quail egg albumen. The improved egg production was not observed during the injection period suggesting that the extra nutrient from albumen was not the cause for the effect on egg production, contrary to the author's original hypothesis. Scott et.al. (1969) reported that most proteins stimulated the formation of specific antibodies when injected into test animals. If the protein was pure, it would result in the formation of a single specific antibody. A mixture of proteins would produce a corresponding number of antibodies. As mentioned earlier in the literature review, Japanese quail egg albumen has at least twelve distinct proteins. The lapse of time before an increased egg production occurred was possibly indicating that antibodies were responsible for the improved egg production. One would postulate that egg albumen being

TABLE 39

EGG PRODUCTION DATA DURING CRD OUTBREAK

Lots	19th Week		20th Week	
	Hen Day %	Hen House %	Hen Day %	Hen House %
A (Cont)	27.1	27.1	57.1	57.1
B (CEA	32.9	32.9	52.8	52.8
C (QEA)	57.1	57.1	57.1	57.1
D (RS)	42.8	34.3	60.7	48.6

a mixture of proteins is an antigen which stimulated the formation of antibodies. Formation of antibodies required time to develop and that might be the reason why during the treatment there was no significant difference in egg production among the lots. The antibodies present in blood serum might play a role in conferring resistance to certain infections. The certain amount of resistance shown by Lot C (QEA) during the CRD outbreak might possibly be due to these antibodies.

The egg production of Lot C remained higher during the initial outbreak of CRD and continued to remain higher than that of the other lots during the height of CRD outbreak. This was considered evidence that possibly the QEA injected

birds had some resistance to CRD infection. As mentioned in the literature review, Hill and Sercarz (1974) reported that Japanese quail egg-white lysozyme and ringed-neck pheasant egg-white lysozyme gave strong responses to all C57 BL/6 mice in their studies on the cellular and humoral antibody and that 90% of the mice were completely unresponsive to chicken egg-white lysozyme, bob-white quail egg lysozyme, guinea-hen egg lysozyme, and pea-fowl egg lysozyme and 10% responded at very low level.

Dang and Visek (1960) injected rats intraperitoneally and chicks subcutaneously thrice weekly with crystallized urease for a 4-week period. In both rats and chicks there were no differences in body weight during the 0 to 4-week injection period. But during the 4-8 week period, the treated chicks gained weight significantly faster ($P < 0.01$) and were more efficient in converting feed to weight gain. The author now realizes that he should have followed up with observations after the injection period for Trials I and II of the chicken experiment, and the 3 trials in the growing swine experiments. Apparently positive results may occur after the termination of egg-white injection as in the layers in Trial III, and the experiments with rats and chicks by Dang and Visek (1960).

Unfortunately a follow-up with the two replications on QEA injections and two control groups was unsuccessful because of the CRD outbreak. The CRD outbreak was on the week when

the hens received the last dosage of QEA injections. These groups suffered severely because of the double stresses, injections of foreign protein and CRD infection. The hens did not have enough time to build up sufficient levels of resistance to CRD infection and so the experiment was terminated.

VI. CONCLUSION

The results obtained in three trials conducted on the growing swine indicate that under these experimental conditions, chicken egg albumen injection has no effect upon the rate of growth of swine. However, low level of egg albumen injection may improve the efficiency in converting feed to weight gain. The amount of labor involved and the difficulty in catching and restraining the pigs for injection may be too involved and economically unfeasible for commercial farmers. There is also the potential risk of bacterial infection if the person involved is careless.

High dosages of albumen injection can cause a deleterious effect on pigs. Albumen injection and egg-white powder fed in the diet of pigs at 1% level slightly improved the dressing percent and ham and loin percent. There was no significant difference in the hemoglobin, total serum protein, ammonia N, urea N, and electrophoretic pattern of plasma proteins.

Experiments conducted on 92 growing chickens failed to show any significant body weight gain upon injection with chicken egg albumen. Male Barred Plymouth Rock birds treated with chicken egg-white showed a slight decrease in the rate of growth as compared to the control birds.

Trial III hens injected with quail egg albumen (QEA) produced significantly more eggs (P 0.01) than those injected with (CEA), Ringer's solution (RS) injected hens and control hens. Out of 107 days of egg production (Hen-house), QEA treated group produced 906 eggs, the control group 803 eggs, the CEA group 797 eggs, and the RS group 779 eggs. Percentage of egg production on a hen-housed and hen day basis was also calculated and found to be highly significant in favor of the QEA group. The QEA group maintained a higher level of egg production during an outbreak of CRD in the flock.

The fact that chickens and pigs did not respond to egg albumen injections during the treatment period nullified the initial hypothesis that the high biological value of egg-white would help to improve the biological value of the protein of the feed and thus produce an increase in growth rate and egg production. The persistently high egg production three weeks after termination of Japanese quail egg albumen injection was suggestive of an immunological effect rather than a nutritional effect from the egg albumen.

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APPENDIX TABLE 40

TRIAL I (a), SWINE

Weekly Records of Performance

Controlled

Pig No.	Sex	10/1	10/8	10/15	10/22	10/29
119-8	F	39.0	43.10	49.44	54.43	58.06
119-12	F	39.9	44.91	51.26	57.15	61.69
239-2	F	34.93	39.46	45.36	49.90	54.43
H15-5	F	34.93	39.46	44.45	49.44	54.43
119-1	M	40.37	44.91	53.07	58.97	63.5
120-2	M	38.56	42.18	49.90	56.25	59.88
241-4	M	33.57	38.10	44.45	49.44	54.43
Total Weight (Kg)		261.27	292.12	337.93	375.58	406.43
Average Wt. (Kg)		37.33	41.73	48.27	53.66	58.06
Total gain period			30.85	45.81	37.65	30.84
Total gain to date			30.85	76.66	114.31	145.15
Ave. da. gain period			0.63	.93	.77	.63
Ave. da. gain to date				.78	.78	.74
Feed Cons. period			77.11	65.77	97.52	108.86
Feed Cons. to date				142.88	240.4	349.27
Ave. da. feed period			1.57	1.34	1.99	2.22
Ave. da. feed to date				1.46	1.63	1.78

APPENDIX TABLE 40 (Con't.)

Pig No. Sex	10/1	10/8	10/15	10/22	10/29
Feed gain period		2.5	1.44	2.59	3.53
Feed gain to date			1.88	2.1	2.4
Pig days period		49	49	49	49
Pig days to date			98	147	196

APPENDIX TABLE 41

TRIAL I (b), SWINE

Weekly Records of Performance

Treated Group

Pig No.	Sex	10/1	10/8	10/15	10/22	10/29
119-9	F	36.29	39.46	45.36	50.35	56.7
120-5	F	36.74	40.37	44.91	48.99	53.07
241-9	F	35.83	39.92	46.28	49.90	54.89
239-3	F	33.11	37.65	43.09	48.08	52.16
119-3	M	40.37	43.55	51.71	58.06	63.5
119-4	M	37.65	42.64	49.44	54.89	58.97
120-1	M	41.28	46.27	50.80	57.15	61.24
241-3	M	35.83	41.73	48.08	52.62	57.15
Total wt. (Kg)		297.11	331.58	379.66	420.03	457.68
Av. wt. (Kg)		37.14	41.45	47.46	52.50	57.21
Total gain period			34.47	48.08	40.37	37.65
Total gain to date				82.56	122.93	106.57
Av. da. gain period			0.62	0.86	0.72	0.67
Av. da. gain to date				0.74	0.73	0.72
Feed cons. period			91.17	76.66	103.0	118.84
Feed cons. to date				167.83	270.8	389.64

APPENDIX TABLE 41 (Con't.)

Pig No. Sex 10/1	10.8	10/15	10/22	10/29
Av. da. feed period	1.63	1.37	1.84	2.12
Av. da feed to date		1.50	1.61	1.74
Feed gain period	1.20	0.72	1.16	1.43
Feed gain to date		0.92	1.0	1.10
Pig days period	56	56	56	56
Pig days to date		112	168	224

APPENDIX TABLE 42

TRIAL II (a), SWINE

Weekly Records of Performance

Control Group

Lot No. 1
Ration 12%, MSU Ration
Date: April 1, 1974
Ping No's 5

Pig No.	Sex	4/1	4/8	4/15	4/22	4/29
H17-9	F	69.40	73.48	77.12	81.19	86.18
H18-1	M	78.01	85.73	88.45	92.98	99.34
204-12	F	84.37	88.45	92.53	96.61	102.97
216-3	M	83.0	87.99	92.98	97.52	101.61
Y29-10	F	81.64	88.45	91.62	96.61	102.06
Total wt. *Kg)		396.45	424.12	442.71	464.94	492.16
Av. wt. *Kg)		79.29	84.82	88.54	92.99	98.43
Total gain period			27.67	18.60	22,23	27.22
Total gain to date				46.27	68.49	95.71
Av. da. gain period			.789	.531	.635	.776
Av. da. gain to date				.662	.653	.685
Feed cons. period			96.16	95.71	97.52	102.97
Feed cons. to date				191.87	289.40	392.36
Av. da. feed period			2.75	2.74	2.79	2.94
Av. da. feed to date				2.74	2.76	2.80

APPENDIX TABLE 42 (Con't.)

Pig No. Sex	4/1	4/8	4/15	4/22	4/29
Feed/lb. gain period		3.48	5.15	4.39	3.78
Feed/lb. gain to date			4.15	4.23	4.10
Pig days period		35	35	35	35
Pig days to date		35	70	105	140

APPENDIX TABLE 43

TRIAL II (b), SWINE

Weekly Records of Performance

Treated Group

Lot No. 2
Ration 12%, MSU Ration
Date: April 1, 1974
Pig No's 6

Pig No.	Sex	4/1	4/8	4/15	4/22	4/29
H17-5	F	83.92	88.91	94.80	99.54	105.24
211-4	F	86.09	91.63	95.26	100.25	105.69
H19-8	F	79.83	84.82	91.17	94.80	100.70
211-5	F	73.94	78.47	83.46	89.36	94.35
H19-6	F	73.03	75.75	80.29	85.28	91.63
H17-8	F	75.75	80.29	83.92	87.09	90.72
Total weight		473.56	499.87	528.90	566.11	588.32
Average weight		78.93	83.33	88.13	92.67	98.07
Total gain period			26.31	29.03	27.22	32.21
Total gain to date				55.34	82.56	114.76
Av. da. gain period			85.73	102.51	87.55	102.97
Av. da gain to date				188.24	275.79	378.76
Feed cons. period			2.04	2.44	2.09	2.45
Feed cons. to date				2.24	2.19	2.25

APPENDIX TABLE 43 (Con't.)

Pig No.	Sex	4/1	4/8	4/15	4/22	4/29
Feed gain, period			3.26	3.53	3.22	3.20
Feed gain to date				3.4	3.34	3.30
Pig days, period			42	42	42	42
Pig days to date			42	84	126	168

APPENDIX TABLE 44

TRIAL III (a), SWINE

Weekly Records of Performance

Lot No: 1
Ration: Basal 10% Protein
Date: August 5, 1974
Pig No's 10

Pig No.	Sex	8/5	8/12	8/19	8/26	9/3	9/9
Y8-4	F	62.14	64.86	63.05	66.23	70.31	76.20
11-6	F	66.68	68.49	71.22	73.03	79.39	82.55
11-7	F	66.23	68.49	70.31	72.58	73.94	75.30
112-1	F	66.68	68.49	71.22	75.30	81.65	84.37
108-3	M	79.83	84.37	89.81	91.63	98.88	105.24
112-2	F	64.77	68.95	70.76	74.39	81.65	87.54
106-6	M	70.31	78.47	78.02	84.37	85.73	(88.45)*
5-9	M	72.12	76.66	80.29	83.92	90.72	94.80
112-4	M	68.49	69.85	75.30	78.93	88.00	93.44
14-2	F	61.69	62.14	64.41	66.68	71.67	77.11
Total wt.		679.95	710.79	734.38	767.04	821.92	775.56
Av. wt.		67.99	71.09	73.44	76.7	82.2	86.27
Total gain, period			30.84	23.59	32.66	54.89	40.37
Total gain to date			30.84	54.43	87.09	141.98	182.35
Av. da. gain, period			.439	.336	.467	.685	.748
Av. da gain to date			.439	.390	.413	.490	.526

APPENDIX TABLE 44 (Con't.)

Pig No. Sex 8/5	8/12	8/19	8/26	9/3	9/9
Feed cons., period	179.17	157.85	122.47	198.68	144.24
Feed cons. to date	179.17	337.02	459.50	658.17	802.42
Av. da. feed, period	2.56	2.25	1.75	2.49	2.67
Av. da. feed to date	2.56	2.41	2.19	2.27	2.33
Feed gain, period	5.8	6.69	3.75	3.62	3.57
Feed gain to date	5.8	6.19	5.28	4.64	4.40
Pig days, period	70	70	70	80	54
Pig days to date	70	140	210	290	344

*Slaughtered on 9/4.

APPENDIX TABLE 45

TRIAL III (b), SWINE

Weekly Records of Performance

Lot No. 2
Ration: Basal 1% egg white powder
Date: August 5, 1974
Pig No's 10

Pig No.	Sex	8/5	8/12	8/19	8/26	9/3	9/9
9-5	F	70.76	74.84	78.47	83.46	87.09	91.17
106-8	M	70.76	75.03	79.83	82.10	85.28	88.91
12-7	M	70.31	73.48	78.93	81.65	88.91	92.53
9-9	M	64.41	68.95	73.94	77.11	84.82	87.80
130-8	M	72.58	75.30	80.29	80.29	83.46	85.28
9-6	F	58.06	62.14	65.32	68.04	73.48	77.11
12-11	M	76.20	81.19	86.64	89.36	96.16	98.88
12-2	F	61.24	64.86	68.50	70.76	76.75	78.47
238-5	M	82.55	87.54	92.53	95.71	100.25	(102.06)*9/6
9-8	M	64.86	72.58	78.93	82.10	89.81	93.90
Total weight		691.74	736.74	783.37	810.58	865.02	794.25
Average weight		69.17	73.62	78.34	81.06	86.50	88.23
Total gain, period			44.45	47.17	27.22	54.43	29.48
Total gain to date			44.45	91.63	118.84	173.28	202.76
Av. da. gain, period			.635	.676	.390	.680	.544
Av. da. gain to date			.635	.653	.567	.599	.590

APPENDIX TABLE 45 (Con't.)

Pig No. Sex	8/5	8/12	8/19	8/26	9/3	9/9
Feed cons., period		188.70	182.8	107.96	196.86	166.47
Feed cons. to date		188.70	371.50	479.46	676.32	842.79
Av. da. feed, period		2.69	2.61	1.54	2.46	3.08
Av. da feed to date		2.69	2.65	2.28	2.33	2.45
Feed gain, period		4.25	3.86	3.97	3.62	5.65
Feed gain to date	4.25	4.25	4.06	4.03	3.90	4.16
Pig days, period		70	70	70	80	54
Pig days to date		70	140	210	290	344

*Slaughtered on 9/4.

APPENDIX TABLE 46

TRIAL III (c), SWINE

Weekly Records of Performance

Lot No. 3
Ration: Basal 10% + Albumen Injection
Date: August 5, 1974
Pigs No's 10

Pig No.	Sex	8/5	8/12	8/19	8/26	9/3	9/9
12-6	M	74.39	76.20	75.30	82.10	87.54	93.0
121-5	F	70.76	75.75	78.47	81.65	85.73	89.81
13-7	M	78.02	83.92	88.45	93.0	98.88	97.98*9/4
13-5	M	68.04	69.85	73.94	74.84	79.83	85.28
8-7	F	63.96	67.13	68.04	67.59[b]	70.76	76.20
6-8	F	61.24	58.97[a]	61.69	60.78[b]	63.50	66.23
121-4	F	70.31	76.66	80.29[c]	82.10	85.28	88.91
18-5	M	68.04	71.22	76.20	81.19	83.46	86.64
19-4	F	63.96	69.85	70.31	75.75	80.29	84.82
19-1	F	62.14	64.86	68.04	72.58	78.93	85.28
Total weight		680.85	714.42	740.73	771.57	814.21	756.15
Average weight		68.09	71.44	74.07	77.16	81.42	84.01
Total gain, period			33.57	26.31	30.84	42.64	40.82
Total gain to date			33.57	59.88	90.72	133.36	174.18
Av. da. gain, period			.481	.376	.439	.535	.758
Av. da. gain to date			.481	.426	.431	.458	.508

APPENDIX TABLE 46 (Con't.)

Pig No. Sex 8/5	8/12	8/19	8/26	9/3	9/9
Feed cons., period	168.74	152.86	103.87	166.11	135.54
Feed cons. to date	168.74	321.60	424.48	591.59	727.12
Av. da. feed, period	2.41	2.18	1.48	2.08	2.51
Av. da. feed to date	2.41	2.30	2.03	2.04	2.11
Feed gain, period	5.03	5.81	3.37	3.90	3.32
Feed gain to date	5.03	5.36	4.69	4.44	4.15
Pig days, period	70	70	70	80	54
Pig days to date	70	140	210	290	344

[a]Lame on right hind leg.

[b]Vomiting at time of injection.

[c]Infection on site of injection.

*Slaughtered on 9/4.

APPENDIX TABLE 47

TRIAL III (d), SWINE

Weekly Records of Performance

Lot No. 4
Ration: 13% finisher
Date: August 5, 1974
Pig No's 10

Pig No.	Sex	8/5	8/12	8/19	8/26	9/3	9/9
138-8	M	66.23	75.48	78.02	84.37	91.65	99.34
18-1	F	72.58	77.57	83.01	84.82	91.63	96.16
18-3	F	60.78	65.32	69.40	69.40	73.03	73.48
18-3	M	71.22	75.75	78.47	82.10	89.91	93.44
18-6	M	61.69	64.41	71.67	75.75	83.92	88.0
138-2	F	64.41	68.49	73.03	76.20	82.56	85.73
138-5	F	61.69	66.68	71.22	71.67	78.47	82.10
112-4	M	74.39	77.57	79.83	84.37	93.90	100.25
18-7	M	68.04	72.58	77.57	81.65	89.81	93.89
138-7	M	73.48	80.29	85.73	88.91	102.06	102.51
Total weight		674.50	722.13	767.94	799.24	876.81	914.91
Average weight		67.45	72.21	76.79	79.92	87.68	91.49
Total gain, period			47.63	45.81	31.30	77.57	38.10
Total gain to date			47.93	93.44	124.74	202.31	240.41
Av. da. gain, period			.680	.653	.449	.971	.544
Av. da. gain to date			.680	.667	.594	.699	.667

APPENDIX TABLE 47 (Con't.)

Pig No. Sex	8/5	8/12	8/19	8/26	9/3	9/9
Feed cons., period		192.78	182.12	102.74	210.92	188.92
Feed cons. to date		192.78	374.90	477.64	688.56	877.49
Av. da. feed, period		2.75	2.60	1.47	2.64	2.70
Av. da. feed to date		2.75	2.68	2.27	2.38	2.44
Feed gain, period		4.05	3.96	3.28	2.72	4.96
Feed gain to date		4.05	4.01	3.83	3.40	3.65
Pig days, period		70	70	70	80	70
Pig days to date		70	140	210	290	360

APPENDIX TABLE 48

TRIAL I, CHICKENS

Weekly Records of Body Weight Gain of Barred Plymouth Female

Control Group

9 Weeks Old	Initial Weight	1st Week	2nd Week	3rd Week	4th Week	Weight Gain
Bird No.	5/1/73 gm	5/8/73 gm	5/15/73 gm	5/22/73 gm	5/29/73 gm	gm
13	970	1115	1264	1420	1500	530
14	846	929	1015	1050	1139	293
15	1019	1119	1242	1325	1409	390
16	994	1120	1228	1355	1417	423.
17	918	1080	1204	1330	1405	487
18	1048	1186	1349	1525	1607	559
Total	5795	6549	7302	8005	8477	2682
Mean	965.8±	1091.5±	1217±	1334.2±	1412.8±	447±

APPENDIX TABLE 48 (Con't.)

Treated Group

9 Weeks Old	Initial Weight	1st Week	2nd Week	3rd Week	4th Week	Weight Gain
Bird No.	5/1/73 gm	5/8/73 gm	5/15/73 gm	5/22/73 gm	5/29/73 gm	gm
31	916	1042	1198	1280	1386	470
32	721	887	1071	1000	1210	489
33	981	1136	1251	1375	1459	478
34	905	1047	1193	1280	1375	470
35	913	1087	1251	1375	1476	563
36	1014	1173	1328	1475	1562	548
Total	5450	6372	7292	7785	8468	3018
Mean	908.3±	1062±	1215.3±	1297.3±	1411.3±	503±

APPENDIX TABLE 49

TRIAL II (a), CHICKENS

Weekly Records of Body Weight Gain of SCWL Male

Control Group

3 Weeks Old

Wing Band No.	7/27/73 wt. gm.	8/3/73 wt. gm.	8/10/73 wt. gm.	8/17/73 wt. gm.	8/24/73 Final wt. gm.	Initial wt. gm.	Weight Gain
6631	204	309	406	554	702	204	498
6612	195	274	340	455	573	195	378
6639	190	285	388	536	681	190	491
6661	194	292	397	517	642	194	448
6605	158	241	321	428	537	158	379
6620	219	319	424	557	697	219	478
6649	218	308	415	558	700	218	482
6607	197	306	426	575	722	197	525
6641	173	256	345	457	558	173	385
6664	201	294	401	524	636	201	435
Total					6448	1949	4499

APPENDIX TABLE 49 (Con't.)

Treated Group

Wing Band No.	7/27/73 wt. gm.	8/3/73 wt. gm.	8/10/73 wt. gm.	8/17/73 wt. gm.	8/24/73 Final wt. gm.	Initial wt. gm.	Weight Gain
6638	203	309	421	589	759	203	556
6614	195	271	351	448	526	195	331
6642	186	275	375	525	657	186	471
6628	199	300	402	535	687	199	488
6647	165	254	339	459	582	165	417
6608	219	316	413	548	684	219	465
6613	207	305	412	546	681	207	474
6660	204	304	409	553	668	204	464
6624	184	281	375	507	607	184	423
6609	161	241	335	454	571	161	410
	Total				6422	1923	4499

APPENDIX TABLE 50

TRIAL II (b), CHICKENS

Weekly Records of Body Weight Gain of SCWL Female

Control Group

3 Weeks Old

Wing Band No.	7/27/73 wt. gm.	8/3/73 wt. gm.	8/10/73 wt. gm.	8/17/73 wt. gm.	8/24/73 Final wt. gm.	Initial wt. gm.	Weight Gain
6632	182	254	332	427	529	182	347
6665	207	282	367	459	566	207	359
6652	185	262	343	448	547	185	362
6650	161	239	307	390	487	161	326
6636	123	178	243	323	418	123	295
6655	204	285	364	457	537	204	333
6606	170	245	327	416	515	170	345
6611	160	233	315	399	490	160	330
6619	188	258	343	436	539	188	351
0000	197	280	353	444	540	197	343
	Total				5168	1777	3391

APPENDIX TABLE 50 (Con't.)

Treated Group

Wing Band No.	7/27/73 wt. gm.	8/3/73 wt. gm.	8/10/73 wt. gm.	8/17/73 wt. gm.	8/24/73 Final wt. gm.	Initial wt. gm.	Weight Gain
6618	180	263	354	475	575	180	395
6658	211	286	367	488	606	211	395
6653	146	214	290	377	463	146	317
6640	204	288	369	483	584	204	380
6627	173	245	320	422	521	173	348
6621	174	246	321	403	481	174	307
6659	159	228	314	410	520	159	361
6637	196	283	378	485	581	196	385
6635	199	289	383	507	632	199	433
6630	196	276	355	454	545	196	349
	Total				5508	1838	3670

APPENDIX TABLE 51

TRIAL II (c), CHICKENS

Weekly Records of Body Weight Gain of Barred Plymouth Rock Male

Control Group

Wing Band No.	7/27/73 wt. gm.	8/3/73 wt. gm.	8/10/73 wt. gm.	8/17/73 wt. gm.	8/24/73 Final wt. gm.	Initial wt. gm.	Weight Gain
6839	313	474	616	707	964	313	651
6827	312	427	564	662	886	312	574
6754	343	491	539	736	964	343	621
6824	324	483	636	742	1028	324	704
6750	303	456	635	704	938	303	635
6822	327	401	609	743	995	327	668
0000	291	467	621	660	803	291	512
6834	348	413	549	777	1039	348	691
6766	295	500	641	663	890	295	595
	Total				8507	2856	5651

APPENDIX TABLE 51 (Con't.)

Treated Group

Wing Band No.	7/27/73 wt. gm.	8/3/73 wt. gm.	8/10/73 wt. gm.	8/17/73 wt. gm.	8/24/73 Final wt. gm.	Initial wt. gm.	Weight Gain
6836	315	465	603	697	690	315	375
6762	312	479	624	730	961	312	649
6745	317	446	574	664	865	317	548
6761	344	478	586	645	836	344	492
6756	325	507	677	786	1072	325	747
6747	336	486	632	733	960	336	624
6746	281	432	566	665	877	281	596
6763	368	541	694	805	1040	368	672
6755	285	408	546	649	831	285	546
	Total				8132	2883	5249

APPENDIX TABLE 52

TRIAL II (d), CHICKENS

Weekly Records of Body Weight Gain of Barred Plymouth Rock Female

Control Group

Wing Band No.	7/30/73 wt. gm.	8/6/73 wt. gm.	8/13/73 wt. gm.	8/20/73 wt. gm.	8/27/73 Final wt. gm.	Initial wt. gm.	Weight Gain
6838	318	437	539	603	819	318	501
6759	308	456	516	564	757	308	449
6743	285	396	504	573	779	285	494
6840	296	366	482	545	721	296	425
6758	252	350	451	519	714	252	462
6753	255	351	460	517	700	255	445
6837	304	416	523	596	783	304	479
0000	269	379	475	542	721	269	452
6767	242	346	459	545	718	242	476
6841	282	365	445	478	651	282	369
6835	288	389	494	579	763	288	475
	Total				8126	3099	5027

APPENDIX TABLE 52 (Con't.)

Treated Group

Wing Band No.	7/30/73 wt. gm.	8/6/73 wt. gm.	8/13/73 wt. gm.	8/20/73 wt. gm.	8/27/73 Final wt. gm.	Initial wt. gm.	Weight Gain
6825	303	437	556	624	866	303	563
6751	308	429	563	653	830	308	522
6829	297	387	491	561	741	297	444
6832	252	359	450	521	685	252	433
6748	255	366	469	547	719	255	464
6826	304	432	552	627	812	304	508
6828	298	418	517	587	764	298	466
6757	272	368	454	505	681	272	409
6833	249	376	480	567	745	249	496
6830	304	437	533	613	826	304	522
6744	286	396	501	677	755	286	469
	Total				8424	3128	5296

APPENDIX TABLE 53

ANALYSIS OF VARIANCE ON PERCENTAGE OF EGG PRODUCTION

Source	df	SS	MS	F
Total	55	2488.48	-	-
Periods	13	1400.44	107.73	2.23*
Treatments	3	413.62	137.83	7.97**
Error	39	674.42	17.30	-

* = P 0.05

** = P 0.01