M.Sc. Thesis June 1969

Effect of Gamma Irradiation Upon Nutrient Stability in Poultry Rations

Christopher L. Cox

Animal Science

ABSTRACT

Six experiments of varying duration were conducted to study the effects of gamma irradiation upon nutrient stability in poultry rations. Three generation studies with chickens were conducted in which irradiated and non-irradiated rations were fed to P_1(parent) stocks and to F_1 and F_2 Generations produced from sib-matings. Dilution of the irradiated starter ration by 25% did not result in minimal or sub-optimal vitamin levels for chick growth. Irradiation of the chick starter ration at dose levels to 3.5 Mrad was not found to significantly affect growth response, feed efficiency or tissue storage of riboflavin and thiamin. Results of TBA Tests upon fat extracted from randomly sampled ration mixtures indicated fat stability was not significantly influenced at irradiation dose levels to 3.5 Mrad. In general, the results of these experiments indicated that chickens of all ages could be fed rations irradiated at the 1 Mrad dose level for disinfection without any adverse effects upon response.

Suggested short title

EFFECT OF IRRADIATION UPON POULTRY RATIONS

Cox.

EFFECT OF GAMMA IRRADIATION UPON NUTRIENT STABILITY IN POULTRY RATIONS

by

Christopher L. Cox

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science.

Department of Animal Science
Macdonald College
McGill University
Montreal

June 1969

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Dr. N. Nikolaiczuk for his interest and assistance offered during the course of the research and in the preparation of this manuscript.

To Professor P. A. Anastassiadis for his helpful suggestions, loan of equipment and assistance with regard to chemical analyses, the author is indebted.

The technical assistance of J. P. Murray and help from A. F. Sefton with statistical analyses is gratefully acknowledged.

Acknowledgement is also made to Atomic Energy of Canada who provided the research grant from which funds for the graduate assistantship were made available and to Robin Hood Flour Mills Ltd. for their co-operation and contribution of feed supplies.

Special appreciation is extended to Mrs. M. Hill for her help and patience during the typing of this manuscript.

TABLE OF CONTENTS

		Page
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	3
	A. The APPLICATION OF RADIATION TO THE TREATMENT OF FOODS AND FEEDS	3
	B. IRRADIATION INDUCED CHEMICAL CHANGES IN FOODS AND FEEDS	6
	(a) Effect on protein	7
	(b) Effect on carbohydrate	8
	(c) Effect on fat and use of Thiobarbituric acid (TBA) in the study of rancidity	9
	(d) Effect on vitamins	12
III.	OBJECT OF RESEARCH	14
IV.	MATERIALS AND METHODS	15
	A. GENERAL	15
	(a) Experimental stocks and experimental procedure	15
	(b) Preparation of rations	15
	(c) Feeding and housing	17
	(d) Tissue collection	18
	B. CHEMICAL ANALYSES	18
	(a) Vitamin extraction	18
	(b) Thiamin	19
	(c) Riboflavin	19
	(d) TBA test	20
	C. STATISTICAL ANALYSES OF DATA	21
V.	RESULTS AND DISCUSSION	22
	A. GENERAL	22
	B. GROWTH TRIALS AND TISSUE ANALYSES	22
	(a) Experiment 1 - P_1 Generation performance and tissue analysis	22
	(b) Experiment 2 - F_1 Generation performance and tissue analysis	30
	(c) Experiment 3 - F_2 Generation performance	33
	(d) Comparison of generation studies	38
	(e) Experiment 4 - Growth trial and tissue analysis	46
	(f) Experiment 5 - Growth trial and tissue analysis	50
	C. TBA TEST - EXPERIMENT 6	53

	Page
VI. SUMMARY AND CONCLUSIONS	55
LITERATURE CITED	57
APPENDIX	

LIST OF TABLES

No.	Title	Page
1.	Chemical composition of ration mixtures used as basal feeds.	16
2.	Effect of irradiation upon a commercial layer ration as measured by egg quality criteria to age 84 weeks - P_1 Generation.	28
3.	Effect of irradiation upon a commercial layer ration as measured by layer reproductive performance - P_1 Generation.	29
4.	Effect of irradiation upon a chick starter ration as measured by tissue vitamin content analyses - P_1 Generation	31
5.	Effect of irradiation upon a commercial layer ration as measured by egg quality criteria to age 34 weeks - F_1 Generation.	34
6.	Effect of irradiation upon a commercial layer ration as measured by layer reproductive performance - F_1 Generation.	35
7.	Effect of irradiation upon a chick starter ration as measured by tissue vitamin content analyses - F_1 Generation.	36
8.	Effect of irradiation upon a commercial layer ration as measured by egg quality criteria to age 34 weeks - F_2 Generation.	39
9.	Effect of irradiation upon a commercial layer ration as measured by layer reproductive performance - F_2 Generation.	40
10.	Effect of irradiation upon a chick starter ration as measured by chick growth response and feed efficiency to age 7 weeks - P_1, F_1 and F_2 Generations.	41

No.	Title	Page
11.	Effect of irradiation upon a chick grower ration as measured by chick growth response and feed efficiency from 7 - 24 weeks of age - P_1, and F_1 Generations and from 7 to 22 weeks of age - F_2 Generation.	42
12.	Effect of irradiation and dilution upon a chick starter ration as measured by chick growth response and feed efficiency to age 5 weeks - Experiment 4.	47
13.	Effect of irradiation and dilution upon a chick starter ration as measured by tissue vitamin content analyses - Experiment 4.	49
14.	Effect of irradiation upon a chick starter ration as measured by chick growth response and feed efficiency to age 5 weeks - Experiment 5.	51
15.	Effect of irradiation upon a chick starter ration as measured by tissue vitamin content analyses - Experiment 5.	52
16.	Effect of irradiation upon fat stability in chick starter and grower rations as measured by the TBA Test - Experiment 6.	54

LIST OF FIGURES

Figure No.	Title	Page
1.	Effect of irradiation upon a commercial layer ration as measured by bird growth response to 84 weeks - P_1 Generation.	24
2.	Effect of irradiation upon a commercial layer ration as measured by egg production to age 84 weeks - P_1 Generation.	25
3.	Effect of irradiation upon a commercial layer ration as measured by feed efficiency in egg production to age 84 weeks - P_1 Generation.	26
4.	Effect of irradiation upon a commercial layer ration as measured by bird growth response to age 34 weeks - P_1, F_1 and F_2 Generations.	43
5.	Effect of irradiation upon a commercial layer ration as measured by egg production to age 34 weeks - P_1, F_1 and F_2 Generations.	44
6.	Effect of irradiation upon a commercial layer ration as measured by feed efficiency in egg production to age 34 weeks - P_1, F_1 and F_2 Generations.	45

INTRODUCTION

In a world where the population increase is clearly outstripping increases in food production, new and improved methods of food preservation continue to receive the attention of countless research workers. One of the newer methods of food preservation, radiation preservation, has shown great potential in this field.

The irradiation of common foodstuffs, through pasteurization and sterilization, is known to cause varying degrees of nutrient degradation. It appears from studies to date that vitamins are generally more sensitive to gamma radiation than other nutrients. Proteins and fats seem to be intermediate in sensitivity while carbohydrates are virtually unaltered at radiation levels usually employed for food preservation.

While radiation of foodstuffs has primarily been concerned with control or elimination of organisms causing food spoilage, the irradiation of poultry rations is concerned more specifically with control of Salmonella and other bacterial organisms. Certain feed ingredients, particularly the animal protein meals, have been found to be contaminated with Salmonella, which gives rise to contaminated mixed feeds. The latter act as vehicles in the transmission of Salmonella to poultry with the eventual production of contaminated poultry products for human consumption.

Improved sanitation procedures and heat treatments have shown some success in reducing contamination during feed manufacture

but have failed to remedy the situation entirely. The present study, of long and short term feeding trials and limited specific nutrient evaluations, aims to provide some information as to the possible effect of irradiation upon nutrient stability in poultry rations.

II. REVIEW OF LITERATURE

A. THE APPLICATION OF RADIATION TO THE TREATMENT OF FOODS AND FEEDS.

Before considering the application of radiation to the treatment of foods and feeds a few of the terms commonly encountered in the literature should be defined by direct quotation (references given).

gamma rays - electromagnetic radiations of short wavelength and great penetrating power produced during the nuclear disintegration of radioactive substances such as Cobalt-60 and Caesium-137 (Young,1964). They are, by far, the most common type used in radiation treatment of food and feed.

rad - radiation absorbed dose equivalent to the absorption of 100 ergs of radiation energy per gram of absorbing material (U.S. Atomic Energy Commission, 1966). 1 Mrad equals 1 million rads.

rep - Roentgen equivalent physical, an obsolete unit of absorbed dose of ionizing radiation with a magnitude of 93 ergs per gram. It has been superseded by the rad (U.S. Atomic Energy Commission, 1966).

radiation disinfection - describes the effect of radiation on the population of pathogenic bacteria; the population is reduced but not eradicated (U.S. Department of Commerce, 1965).

radiation sterilization - the reduction of the number of contaminating organisms in food by ionizing radiation to such an extent that none are detectable in treated food by any recognised method, no matter how long or under what conditions the food is stored in the absence of recontamination (Ministry of Health, 1964).

radiation pasteurization - the control by radiation of the spoilage organisms and pathogens which are most likely to be troublesome or dangerous in food that is to be irradiated (Ministry of Health, 1964).

radiation disinfestation - the application of doses of ionizing radiation sufficient to control infestation of foods by insects and parasites (U.S. Department of Commerce, 1965).

radiation sprout inhibition - the application of radiation to vegetables subject to sprouting in order to inhibit or remove the ability to sprout (U.S. Department of Commerce, 1965).

The first patent for the use of ionizing radiations to preserve food was issued in France in 1930. Serious application of research on the possibilities of irradiation for food preservation was not undertaken however until the early 1940's in the United States. (U. S. Department of Commerce, 1965).

From this limited beginning, research on the potential of radiation for the treatment of foods and feeds has expanded intensively in many nations of the world.

Doses of radiation (4.5 - 5.6 Mrad) for the sterilization of meat and meat products has been shown to increase shelf and storage life of these products indefinitely provided they are not recontaminated. Low levels of irradiation (0.005 - 0.010 Mrads) for sprout inhibition of potatoes has been highly effective and has been approved in Canada by the Food and Drug Directorate.

Considerable work on the control of Salmonella organisms in certain foods and animal feeds has been carried on in the United States, the United Kingdom, the Netherlands, and Canada. Results indicate that a dose of 0.5 - 1.0 Mrad of gamma radiation controls or destroys all known types of Salmonella (International Atomic Energy Agency, 1963). Animal feeds and ingredients were considered a major source of Salmonella contamination (Thornley, 1964).

Mossel et al.(1967) indicated a combination of improved sanitation, pelleting at the highest possible temperature and if still required, terminal low-dose irradiation (0.5 - 1.0 Mrad) of bagged feeds would be a promising approach to the manufacture of Salmonella - free feeds.

Leistner et al. (1961), upon studying feeds, found a drastic difference between incidence of Salmonella in commercial feed samples obtained from farms (13 percent) and samples of rendered animal by-products (61 percent).

Bryan et al. (1968) carried out an extensive study on contributing sources of Salmonella in turkey products and reported feed (9 percent) and feed ingredients (11 percent) as sources of Salmonella.

Ley et al. (1963) observed marked variation in radiation resistance between different Salmonella serotypes, S. typhimurium being consistently the most resistant in the food and feed ingredients studied. They also demonstrated the extent to which the nature of the medium influences the resistance of these organisms to gamma radiation.

Idziak and Incze (1968) stated Salmonella species with increased irradiation resistance would probably not occur after radiation treatment of fresh poultry.

Appart from the positive effects of gamma irradiation already noted, other effects and chemical changes in food products have been observed which may have application in poultry feed irradiation.

B. IRRADIATION INDUCED CHEMICAL CHANGES IN FOODS AND FEEDS

Although higher irradiation doses (4 - 6 Mrad) used in food preservation may produce changes in colour, texture, flavour, and odour, generally these changes have been small. Improved control of irradiation dose, temperature, atmosphere, and storage time after irradiation has all but eliminated these problems. It is not known, as yet, if the palatability of poultry rations would be reduced at the levels being proposed for disinfection (0.5 - 1.0 Mrad).

Reported chemical changes induced by irradiation in protein, carbohydrate, fat and vitamin content of foods and feeds have been variable.

With the possible exception of vitamins, nutrient content degradation of foods which are generally of higher moisture content has been minor particularly at low irradiation dose (0.5 - 1.0 Mrad) levels. It is possible that nutrient degradation in irradiated poultry rations may be significantly less than that of common fooods due to a much lower moisture content.

(a) Effect on proteins

When purified proteins are irradiated, alterations in their physical properties usually occur. The Ministry of Health (1964) speculated that such alterations could result from the fission, and reformation in a different way, of only a few chemical bonds, perhaps only one, in each protein molecule, and need involve no change in the constituent amino acids. The Ministry of Health (1964) also reported that amino acids in combined form in proteins appear to be less sensitive to the degrading actions of radiation than they are in the free state.

Metta and Johnson (1959) reported no nutritional damage to the proteins of corn and wheat gluten when irradiated at 2.8 and 9.3 Mrad levels.

Sheffner et al. (1957) observed no significant irradiation destruction of essential amino acids in milk, turkey or beef and only a small loss of cystine in pork.

Read (1960) stated that irradiation (2.8 and 5.6 Mrad) may have improved the utilization of soybean protein by inactivating the

trypsin inhibitor but higher doses decreased protein quality.

Only minimal effect of irradiation (2.5 Mrad) on the amino acid content of haddock fillets was reported by Brooke et al. (1966).

Kennedy (1965) found no effect on protein concentrates when irradiated at 5.0 Mrad but a 26% loss in nutritive value of wheat gluten was observed. This loss was mainly due to degradation of methionine.

That doses of gamma-radiation up to 0.5 Mrad have no effect on the protein of wheat was noted by Cornwell (1959).

Metta and Johnson (1959) demonstrated that irradiation appears to affect the digestibility of proteins in a manner comparable with heat treatments. Ferrando et al.(1968) observed that sterilization of rat diets, with irradiation (4.5 Mrad) or by autoclaving, resulted in significantly higher protein efficiency and less loss of some amino acids with the irradiated treatment.

(b) Effect on carbohydrates

Most studies on the influence of irradiation on purified carbohydrates have been carried out with dilute water solutions of carbohydrates treated with large doses, up to 100 Mrad, of radiation. The Ministry of Health (1964) reported that when foodstuffs have been irradiated under such conditions the main effect of the carbohydrate present has usually been a very slight increase in the amount of free sugar.

Read (1960) found a decrease in the digestibility of starch by rats after irradiation at 2.78 and 5.6 Mrad doses but this decrease

was reduced by the presence of irradiated fat. Kertesz et al. (1959) in a study involving starch and starch fractions observed that amylase and amylopectin are degraded by gamma radiation (1.5 Mrad) in the same manner, whether irradiated together in the form of starch or as separate fractions.

The few references available in the literature tend to be conflicting as to whether any significant effects can or cannot be demonstrated upon irradiation of carbohydrates. Generally, high moisture foods suffer more serious degradation than low moisture foods. It also appears that other food components afford some measure of protection to the carbohydrate fraction. Since poultry rations are of relatively low moisture content the effect of irradiation upon the carbohydrate component may be insignificant especially at the low level (1 Mrad) being considered for disinfection.

(c) Effect on fat and use of Thiobarbituric acid (TBA) in the study of rancidity

The irradiation of fats induces changes which are similar to those which result from autoxidation but as might be expected the irradiation of purified fats results in greater changes than are seen in the fat contained in food irradiated under similar conditions (Ministry of Health, 1964). The changes which result such as peroxide, polymer, and carbonyl compound formation appear related to the size of the irradiation dose applied.

Ritchey and Richardson (1960) using growth and mortality of chicks as criteria, showed that diets containing 10 percent control

and irradiated (2.8 Mrad) soybean and corn oils were essentially equal in nutritive value and storage for 3 weeks at ambient temperatures did not decrease their value. Irradiated diets containing beef and pork fatty tissues however produced in poor growth and high mortality unless an antioxidant was added to the rations before storage and feeding.

Schrieber and Nasset (1959) concluded that irradiation of lard was detrimental to its digestion in the dog due to the formation of peroxides.

Sribney et al. (1955) found that oxidation changes such as peroxide, carbonyl and free fatty acid formation, are not marked during irradiation and subsequent storage, if the presence of oxygen is minimized. Similarly, Morgan (1958) stated that the production of peroxides is completely prevented in the absence of oxygen.

Green and Watts (1966) observed that lipid oxidation in cooked meats was inhibited by radiation at 4.8 Mrad. This inhibition became more pronounced upon storage in sealed cans and appeared to be due to a combination of antioxidant development and reaction of oxidation products. Tipples and Norris (1965) noted a similar trend upon storage of irradiated wheat flour but to a lesser degree.

In the present study, the TBA test was used in an attempt to determine the effect of irradiation upon fats.

A colour reaction between thiobarbituric acid (TBA) and a number of aromatic aldehydes was noted by Dox and Plaisance as early as 1916. Upon addition of TBA to incubated tissues, Bernheim et al. (1948) found that the resulting colour was due to a product of the oxidation

of unsaturated fatty acids, particularly linolenic acid.

Patton and Kurtz (1951) subjected a large number of compounds, in addition to those studied by Wilbur et al. (1949), to the TBA reagent and in so doing developed a test which could be applied to oxidized milk fat. Their evidence indicated that the colours produced with malonic dialdehyde (M.A.) and oxidized milk fat were identical.

The TBA test was used by Tarladgis and Watts (1960) to study M.A. production during the oxidation of pure unsaturated fatty acids under controlled conditions. Cain et al. (1956) and Green and Watts (1966) used the TBA test to study lipid oxidation in irradiated cooked beef.

Biggs and Bryant (1953) used a modified TBA test to detect oxidation rancidity in milk and milk products and concluded that this test was more sensitive than conventional tests such as iodine value and Kries test. Similarly, Caldwell and Grogy (1955) found the TBA test in cereal and baked products to provide a more sensitive and reproducible means of detecting and recording incipient oxidative rancidity than the peroxide value technique.

Use of a standard curve as a means of reporting results was proposed by Sinnhuber and Yu (1958). 1, 1, 3, 3 - tetraethoxy propane (1, 1, 3, 3 - TEP), on hydrolysis will yield 1 mole of MA which reacts quantitatively with TBA. They suggested that the term TBA number or mg. of MA per 1000 g of material be used to express results. Tarladgis et al. (1960), Tarladgis et al. (1964), Smith (1966) and Lees (1967) have made use of this proposed standard curve.

Evidence by many workers to suggest that TBA reactive substances and MA are the same or very similar has met with some argument. Saslow and Warandekar (1965) reported that studies on extracts of irradiated fatty acid showed that none of the TBA reactive substances was MA while Kwon et al. (1965) suggested the whole subject warrants careful re-evaluation.

(a) Effect on vitamins

A review of the literature revealed wide differences in the extent to which different vitamins are affected by irradiation. When foods are exposed to ionizing radiations losses of some vitamins may occur, the extent of the loss depending on the vitamin, the food, the dose and the environment (Ministry of Health, 1964).

Richardson et al. (1958) found no effect on water-soluble vitamins, choline, folic acid, thiamin, riboflavin, pyridozine, and pantothenic acid resulted when a chick ration was irradiated with gamma rays. Coates et al. (1963) reported that less loss of fat-soluble vitamins occurred in vacuum-packed irradiated chick diets than in air-packed irradiated diets. They further reported that a stabilized preparation of Vitamin A suffered less destruction upon irradiation than did vitamin A acetate. Ferrando et al. (1968) reported a 14 to 17 percent loss of vitamin A from rat diets sterilized with either 4.5 Mrad of gamma radiation or autoclaved at 115° for 80 minutes.

Many workers (Brin et al., 1961; Alexander et al., 1956; Groninger and Tappel, 1957) have shown that thiamin was significantly

destroyed in meats by gamma irradiation. Wilson (1959) confirmed these observations but discovered that this destruction could be prevented by freezing at -75°C before and during irradiation.

Kennedy (1965) studied the effect of irradiation on B-complex vitamins in frozen whole eggs and found no change in pantothenic acid, biotin and riboflavin but did report a 61 percent loss of thiamin at a dose of 5.0 Mrads. Similar analyses on Manitoba wheat irradiated at 0.002 Mrad revealed no effect except for a slight loss of pantothenic acid content.

Metta et al. (1959) and Johnson et al.(1960) have reported significant decreases in the vitamin K content of irradiated meat. Richardson et al. (1956) using a chick bioassay, found the loss of vitamin K activity to be comparatively small when a natural food was irradiated. The natural foods utilized in the study by Richardson did not include meat, however.

III. OBJECT OF RESEARCH

This research project was undertaken to provide further information upon the feasibility of radiation disinfection of poultry rations and particularly to study the effect of gamma irradiation upon nutrient stability in poultry rations as follows:

(1) Biological assessment of ration changes using:
   a) Long-term generation studies.
   b) Short-term chick feeding trial to study effect of ration dilution plus standard irradiation dose (1 Mrad).
   c) Short-term chick feeding trial to study effect of increased irradiation intensity doses (1.0, 2.25, and 3.5 Mrad).

(2) Tissue vitamin content assays for thiamin and riboflavin.

(3) TBA test to study possible induced oxidative rancidity.

IV. MATERIALS AND METHODS

A. GENERAL

(a) Experimental stock and experimental procedure

The P_1(parent) stocks were obtained from the Macdonald College No. 2 strain, a Single Comb White Leghorn, small egg line strain. Experiment 1 initiated this study with the P_1(parent generation which was fed irradiated poultry rations for 84 weeks. The successive F_1 and F_2 generations utilized in Experiments 2 and 3 respectively were obtained through pen sib matings originating from this P_1(parent) generation. Studies conducted in Experiments 2 and 3 were similar to those in Experiment 1 except these generations were reared to sexual maturity viz. 34 weeks at which time eggs were collected and incubated to produce an off-spring generation. Chicks in Experiment 4 were surplus cockerels from a commercial Single Comb White Leghorn strain obtained from a local hatchery. This experiment was a 5 week growth trial in which diluted irradiated rations were fed to study vitamin storage in body tissues. The unsexed chick groups in Experiment 5 were surplus chicks hatched at Macdonald College from Macdonald College No. 3 strain female crossed with commercial strain cockerels. Rations irradiated at higher dose levels were fed in this 5 week growth trial for further study of vitamin storage in body tissues.

(b) Preparation of rations

All basal rations (20% Starter, 17% Grower and 15% Layer) used in these studies were supplied by a commercial feed company. The chemical composition of rations used is presented in Table 1. With the exception of Experiment 4, no changes were made to the above rations. In Experiment 4 an attempt was made to dilute the vitamin content of the commercial ration by using a dilution factor of 25 percent i.e. 3 parts regular

Table 1. Calculated composition of ration mixtures used as basal feeds.

	Starter	Grower	Layer
Crude Protein (%)	20.0	17.0	15.0
Crude Fat (%)	3.0	3.0	3.0
Crude Fibre (%)	5.0	8.0	6.0
Riboflavin (mg./Kg.)	6.69	6.36	5.68
Thiamin (mg./Kg.)	5.06	6.86	6.29
Metabolizable Energy (Kcal/Kg.)	2669	2526	2541
Productive Energy (Kcal/Kg.)	1914	1863	1912

ration plus 1 part cereal protein-mineral mix. This cereal protein-mineral mix was supplied by a commercial feed mill and was equivalent to a 16 percent layer ration except that no vitamin premix was added.

The irradiation treatment of rations was carried out by Atomic Energy of Canada Limited using a Cobalt-60 gamma irradiator. The 50 pound bags of feed supplied at 2 month intervals and in batches of similar composition were divided into two equal lots. The lot to be irradiated was emptied into fifty pound-size plastic bags and sealed. These plastic bags of feed were then placed in corrugated cardboard container boxes which were subsequently sealed with masking tape. The regular irradiation treatment of each batch supplied was a 1 Mrad dose level. In Experiment 5 a portion of the ration allotment received a 3.5 Mrad dose. An attempt, in theory, to prepare a ration irradiated at 2.25 Mrad was done simply by diluting a portion of the 3.5 Mrad irradiated ration with non-irradiated control ration.

All rations were stored in a separate, unheated feed room upon arrival at Macdonald College. The periods of storage varied and rations were used as required.

(c) Feeding and housing

All chicks were started in battery brooders under similar environmental conditions. Groups were separated according to treatment and given feed and water ad libitum. Chicks of Experiment 4 and 5 were on test for 5 weeks and thus received only 20 percent starter ration. In Experiment 1, 2 and 3 chicks were raised until 7 weeks of age on the starter ration. In all cases, body weight and feed consumption data were taken weekly, while mortality and other relevant observations were recorded continuously.

At 7 weeks of age, the different treatment groups of the generation studies were placed in floor pens and changed to a grower ration. Body weight and feed consumption data were collected bi-weekly. Feeding of a layer ration began at approximately 24 weeks of age in Experiments

1 and 2 and at 22 weeks of age in Experiment 3. Conditions in the floor pens were similar and each pen was equipped with one tube-type feeder and one automatic waterer. Care was exercised to keep feed and water spillage and wastage at a minimum.

(d) Tissue collection

Muscle and liver tissue from birds in Experiment 1, 2, 4 and 5 was analyzed for thiamin and riboflavin content. A total of 60 birds from Experiments 1 and 4, 20 birds from Experiment 2 and 40 birds from Experiment 5 were examined. All birds were randomly selected and sacrificed during the growing period. Following a sufficient period to allow whole body cooling at room temperature, the breast and liver of each bird was removed, washed in cold water, dried lightly by rolling in absorbent paper, and wrapped in Saran Wrap. The tissue was then frozen and held in cold storage at $0^{o}C$ until time of analysis.

B. CHEMICAL ANALYSES

(a) Vitamin extraction

The procedure for vitamin extraction from the wet tissue was the same for both thiamin and riboflavin. Five grams of muscle tissue or 2 grams of liver tissue were weighed and mixed with approximately 25 ml. of water in a 50 ml. Virtis Model 45 homogenizer flask. After homogenization the slurry was adjusted to pH 1.5 with 8NHCl. Pepsin enzyme was then added (.004gm./gm. tissue) and the tissue solution was placed in a water bath at $37^{o}C$ for approximately 24 hours until complete digestion had occurred. Following digestion the solution was adjusted to pH 4 with sodium acetate

or sodium hydroxide solutions and then centrifuged. The supernatant was decanted and the precipitate washed twice with water. The pooled solution was then made up to 50 ml. with a 1:1 solution of water and 2.5 M sodium acetate and stored in the cold and dark until analysis.

(b) Thiamin

Thiamin content of the muscle and liver extracts was determined by the method described in the Macdonald College Department of Chemistry laboratory manual, number 451a, see Appendix I. The thiamin content of a gram of tissue was calculated according to the following equations:

$$\gamma \text{ thiamin/gm. muscle} = \frac{E - EB}{S - SB} \times 20 \; ; \; \gamma \text{ thiamin/gm. liver} = \frac{E - EB}{S - SB} \times 50$$

where E = galvanometer reading for solution from aliquot of extract.

EB = galvanometer reading for blank of previous reading.

S = galvanometer reading for standard solution containing 1γ/ml.

SB = galvanometer reading for blank of previous reading.

All readings were taken on a Coleman Fluorimeter Model 12.

(c) Riboflavin

Riboflavin content of the muscle and liver extracts was determined by the method described in the Macdonald College Department of Chemistry laboratory manual, number 451a, see Appendix II. The riboflavin content of a gram of tissue was calculated according to the following equations:

$$\text{ug. riboflavin/gm. muscle} = \frac{A - C}{B - A} \times 2 \; ; \; \text{ug. riboflavin/gm. liver} = \frac{A - C}{B - A} \times 5$$

where A = galvanometer reading measuring fluorescence in sample extract

B = A + standard amount of riboflavin added

C = Fluorescence in A not due to riboflavin

All readings were taken on a Coleman Fluorimeter Model 12.

(d) TBA test

Randomly sampled rations were used in a lipid extraction procedure described by Bligh and Dyer (1959) - see Appendix III. The TBA test as described by Tarladgis et al. (1960) was used to determine oxidative rancidity.

The TBA test was carried out using 1 gram of sample dissolved in 5 ml. of benzene and 5 ml. of TBA reagent. A reagent blank was prepared using 5 ml. of TBA reagent, 3 ml. of 95% ethanol and 2 ml. of distilled water. The reagent blank was used to adjust the instrument to 100% T. The TBA reagent used was made up as follows: 0.67 gm. of TBA dissolved in 140 ml. of distilled water and 60 ml. of glacial acetic acid. The flask was held in hot water to facilitate solution.

A heating time of 30 minutes was used for the reaction. Maximum absorbency was read on a Bausch and Lomb Spectrophotometer Model 505 at 530 mu.

A standard curve was prepared as proposed by Sinnhuber and Yu (1958), using known amounts of 1, 1, 3, 3 - TEP. On hydrolysis, one mole of 1, 1, 3, 3 - TEP produces one mole of M.A.

C. STATISTICAL ANALYSIS OF DATA

Analyses of variance were carried out on rate of gain, feed efficiency, Haugh Unit values for egg albumen quality, tissue vitamin content and TBA test results. The forms of analysis used for tissue vitamin content in Experiment 1, 2 and 5 and the TBA test of Experiment 6 were similar. The form of analysis used for rate of gain and feed efficiency data was similar in all cases except in Experiment 4 and for Haugh Unit data summaries. Since no replications were made in Experiment 4 the rate of gain, feed efficiency and tissue vitamin content data required a slightly different form of analysis from the two basic forms used in the other experiments. The form used for Haugh Unit data summaries varied slightly from that used on rate of gain and feed efficiency data. The two basic forms of analysis are illustrated in Appendix IV.

Significant differences were tested with the Duncan's Multiple Range Test as outlined by Steel and Torrie (1960).

V. RESULTS AND DISCUSSION

A. GENERAL

The purpose of Experiment 1 was to study the effect of feeding irradiated rations to a generation of birds for an extended period of time, viz. 84 weeks. Experiments 2 and 3 involved studies on the F_1 and F_2 generations which were carried to 34 weeks of age. Criteria, such as growth response, feed efficiency, egg production, egg quality, reproductive performance, and riboflavin and thiamin content of muscle and liver tissue, are discussed. A brief comparison is drawn between the performances of these three generations.

Experiment 4 and 5 were short term growth trials established to study the effect of diluting irradiated rations and to study the effect of irradiating rations at higher dose levels, respectively. Growth response, feed efficiency, and tissue riboflavin and thiamin content criteria are discussed.

Finally, in Experiment 6, the results of the TBA test, which was utilized to study possible induction of fat rancidity by ration irradiation, are discussed.

B. GROWTH TRIALS AND TISSUE ANALYSES

(a) Experiment 1 - P_1 (parent) Generation performance and tissue analysis

The results of the brooding period are presented in Table 10. Growth response of the non-irradiated groups was slightly better than that of the irradiated groups but their feed efficiency was inferior to that of the irradiated groups. These small differences were not statistically significant however. A sacrifice of chicks was made at

the end of the brooding period to equalize group numbers to 25 females and 10 males for the next growth phase and to provide analytical material for tissue vitamin composition studies. The results of the next growth phase to age 24 weeks are summarized in Table 11. Growth response and feed efficiency of all groups was variable. Again, these small differences probably are not meaningful since analyses of variance on rate of gain and feed effeciency from week 8 to 14 during which no mortality occurred, presented in appendix Tables i - iv, indicated differences due to treatment were not significant. The results of postmortem examinations of all birds which died during the first 24 weeks under study revealed no gross visible lesions or evidence to suggest an effect due to ration treatment. The higher early mortality of control group B may be due to weaker chicks upon hatching at the beginning of the trial but no definite explanation can be given.

All groups were culled to 20 females and 5 males at 28 weeks of age. Following egg collection and incubation to produce the second generation, the replicate of each group was discarded to meet recommended floor space requirements and to conserve feed supplies. Body weight, egg production and feed efficiency data for the laying period are summarized in Figures 1, 2 and 3. Except for the slightly heavier males of the irradiated group, the small differences between the two treatment groups in body weight do not appear meaningful and/or attributable to irradiation of the ration (Figure 1). Egg production by the control group was almost consistently better than that of the irradiated group for the duration of the laying period (Figure 2.). As would be expected, this higher rate

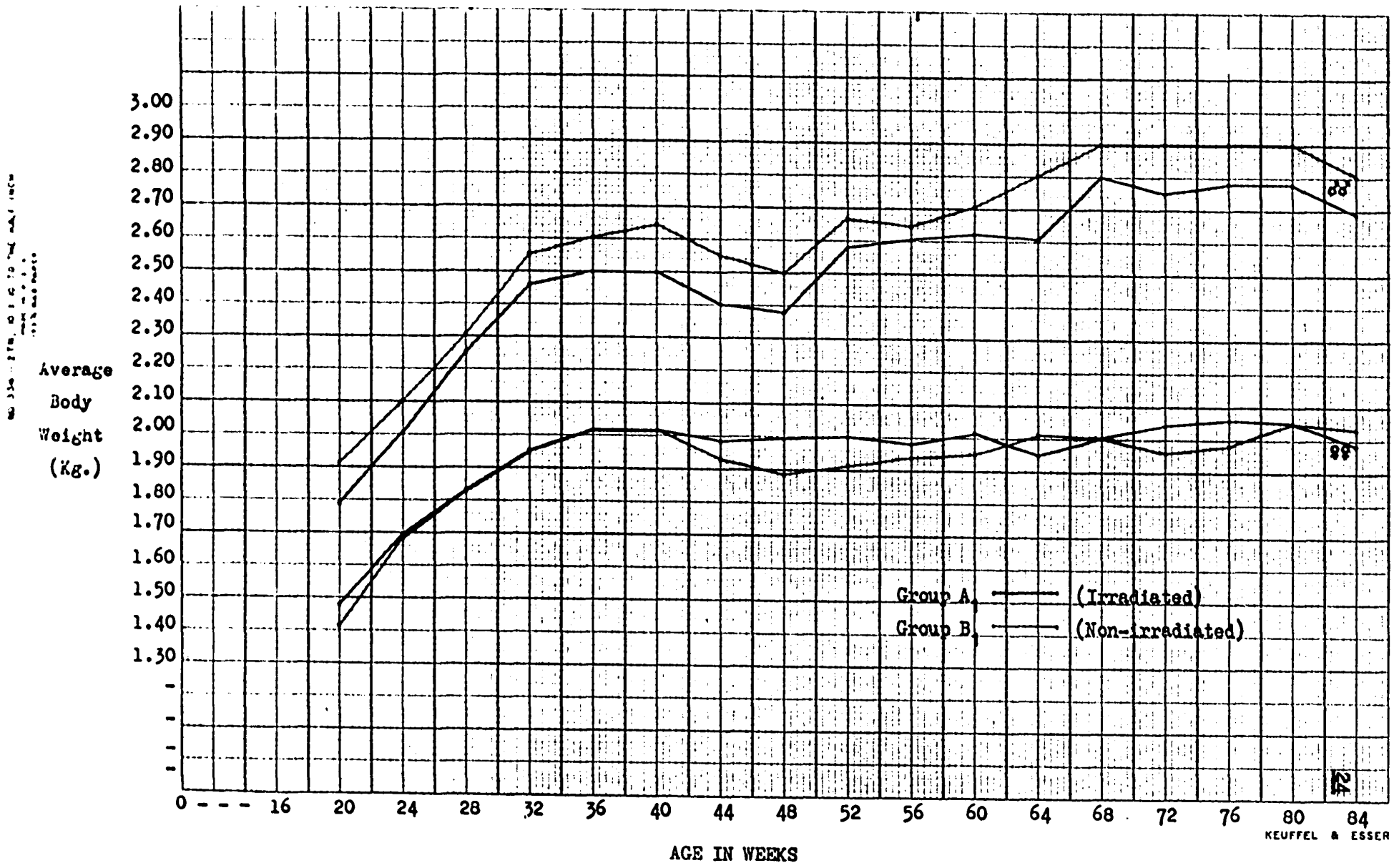

Figure 1. Effect of irradiation upon a commercial layer ration as measured by bird growth response to age 84 weeks - P_1 Generation.

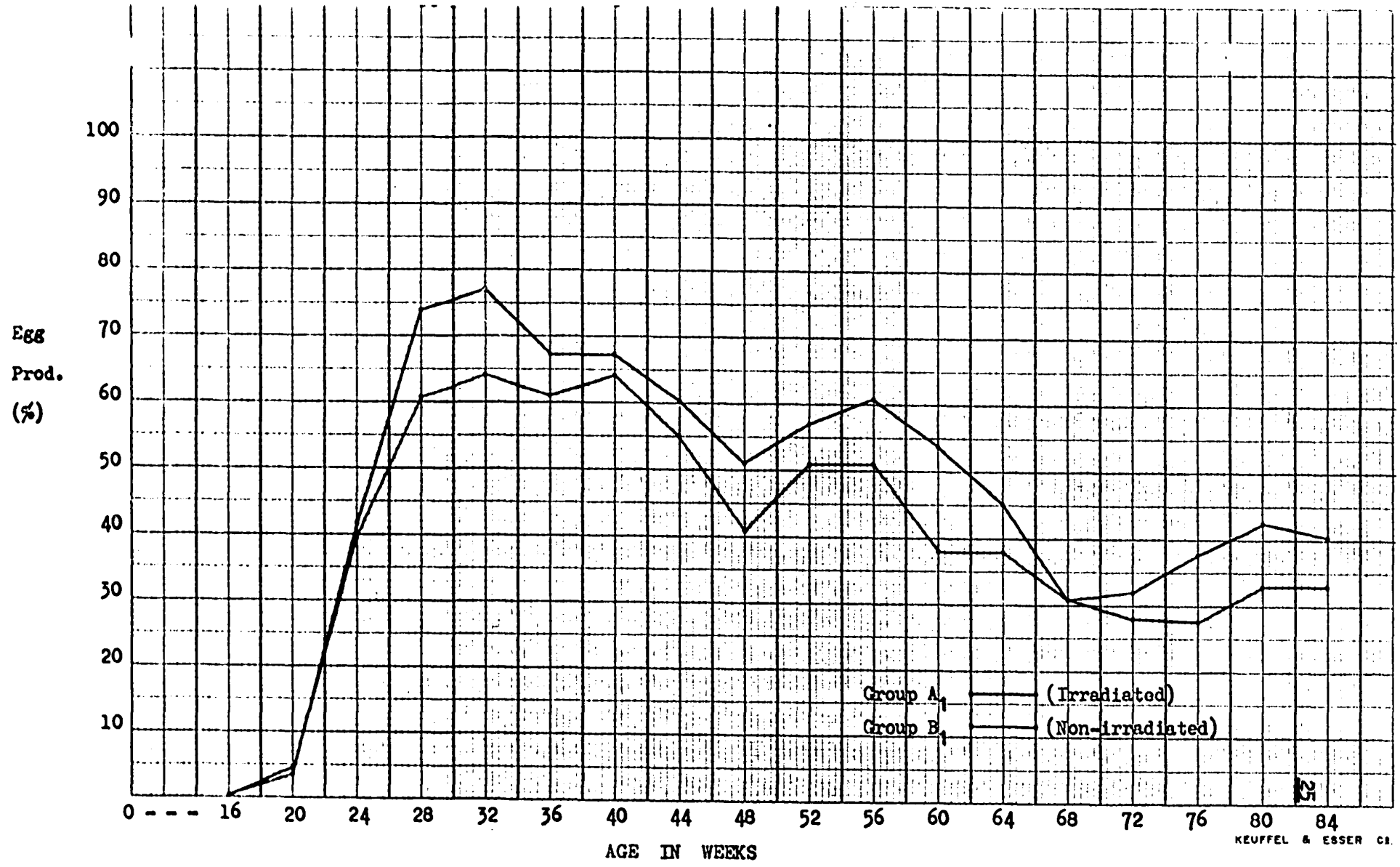

Figure 2. Effect of irradiation upon a commercial layer ration as measured by egg production to age 84 weeks - P_1 Generation.

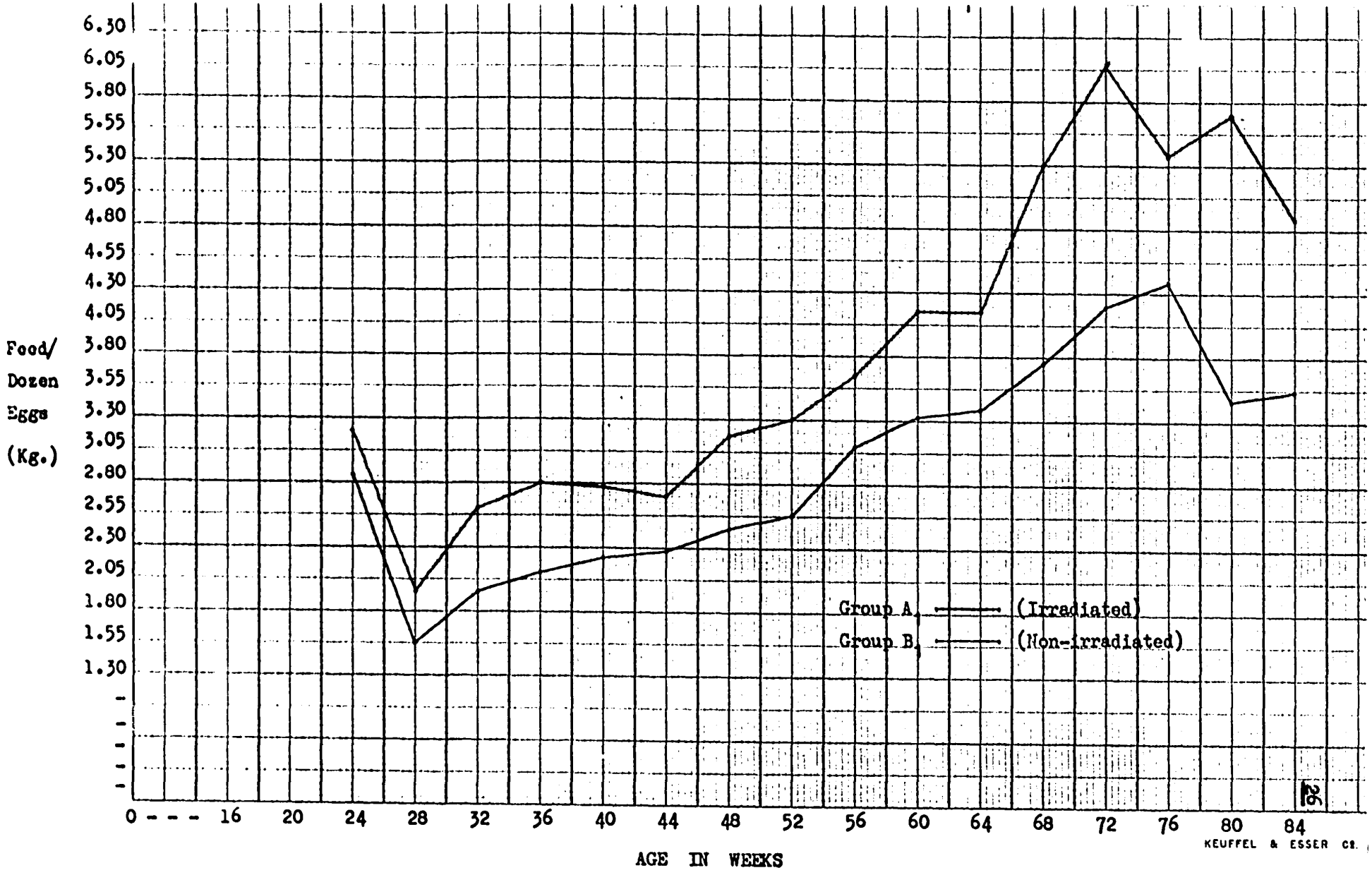

Figure 3. Effect of irradiation upon a commercial layer ration as measured by feed efficiency in egg production to age 84 weeks - P_1 Generation.

of egg production also resulted in the control group having a superior feed efficiency for the laying period (Figure 3). Reduced body weight of the males and egg production of the females at 48 weeks was primarily the result of a severe fowl mite infestation. The birds responded favourably after treatment. Several birds were observed to be undergoing a natural annual moult at approximately 68 to 72 weeks of age, thus egg production was reduced. As expected, egg production declined significantly in both groups during the second year of age. Mortality, which was slightly higher in the irradiated group, particularly during the later stage of production, probably contributed to the inferior performance of this group, especially with respect to feed efficiency. It seems that variability between birds could be a significant factor producing this lower performance since bird numbers were reduced during the laying period. Diagnostic gross examinations on all birds which died during the laying period indicated no carcinogenic effect due to extended feeding of irradiated rations. This finding is in agreement with a study by Burns et al. (1956) who carried a generation of chickens on irradiated and non-irradiated diets to 54 weeks of age.

A summary of the egg quality data are presented in Table 2. An analysis of variance on Haugh Unit values is presented in appendix Table v. This analysis of variance indicated no significant differences in egg quality due to ration treatment. Decreasing quality is primarily a result of seasonal and age effects. Prior to 28 weeks of age, 125 eggs for each replicated treatment group were collected and incubated. A summary of the observations relative to reproductive performance is presented in Table 3.

Table 2. Effect of irradiation upon a commercial layer ration as measured by egg quality criteria to age 84 weeks - P_1 Generation.

Lot No.	Period Weeks	Ration Treatment	Number Layers	Egg Quality* Size (gms.)	Sp.Gr**	Haugh Units	Yolk Color
A_1	32-36	Irradiated	20	50.7	1.091	82.9	7
	36-40		20	49.8	1.090	81.3	6
	40-44		20	50.0	1.089	69.2	6
	44-48		19	50.0	1.089	69.2	6
	48-52		17	54.8	1.080	80.0	6
	52-56		16	55.7	1.084	77.9	6
	56-60		16	56.4	1.088	80.1	6
	60-64		16	54.0	1.084	48.0	5.7
	64-68		16	54.2	1.084	62.4	5.0
	68-72		15	52.8	1.083	57.8	5.3
	72-76		15	53.4	1.084	31.8	6.0
	76-80		15	56.9	1.084	39.5	5.3
	80-84		15	55.4	1.086	22.5	5.9
Mean				53.4	1.086	61.7	5.9
B_1	32-36	Non-irradiated	20	52.5	1.088	87.2	7
	36-40		20	54.5	1.090	88.2	6
	40-44		20	53.1	1.087	78.6	6
	44-48		20	50.0	1.089	69.2	6
	48-52		20	55.7	1.089	75.0	6
	52-56		20	56.4	1.085	81.5	6
	56-60		20	57.8	1.086	77.9	6
	60-64		20	55.4	1.078	55.4	5.3
	64-68		19	58.0	1.079	63.4	5.5
	68-72		19	59.6	1.078	56.0	5.8
	72-76		19	59.1	1.080	39.6	6.1
	76-80		18	61.6	1.080	35.6	6.5
	80-84		18	59.7	1.079	31.6	6.8
Mean				56.4	1.084	64.6	6.1

* All figures are an average of 10 eggs per group per period.

** Specific Gravity

Table 3. Effect of irradiation upon a commercial layer ration as measured by layer reproductive performance - P_1 Generation.

Lot No.	Ration Treatment	Egg Incubation Observations					
		Eggs Set	Infertile	Blood Rings	Dead Germs	Dead in Shell	% Hatch of Fertile Eggs Set
A	Irradiated	125	9	6	29	18	54.3
A_1	"	125	19	4	21	10	67.0
Mean %			11.2	4.0	20.0	11.2	60.7
B	Non-irradiated	125	9	11	24	15	56.9
B_1	"	125	8	12	18	7	68.4
Mean %			6.8	9.2	16.8	8.8	62.6
% Difference - Irrad. vs. Non-irrad.			(+)64.7	(-)56.5	(+)19.1	(+)27.3	(-)3.0

The narrow, but consistent differences favouring the non-irradiated ration group including a higher hatchability of eggs set, may suggest some adverse effect due to feed irradiation but the limited data indicates the need for further study of this depression in layer reproductive performance.

The results of tissue vitamin content analyses are summarized in Table 4. The riboflavin and thiamin content of tissue from birds being fed irradiated feed was slightly higher than that of tissue from the control birds. Growth rate and feed consumption, however, may be an influencing factor in this slightly higher tissue vitamin content. Analyses of variance of these data, presented in appendix Tables vi - ix, indicate no significant differences due to ration treatment.

(b) Experiment 2 - F_1 Generation performance and tissue analysis.

The data for the brooding period of birds involved in Experiment 2 are summarized and presented in Table 10. Growth response and feed efficiency of the irradiated groups was superior to that of the non-irradiated groups but differences are small and may not be a result of ration treatment. Early mortality for all groups was relatively high and the chicks appeared very weak at the beginning of the trial. Small egg size of the parent breeders when eggs were collected for hatching was the primary factor involved, resulting in small, weak chicks for this generation. It is also conceivable that inbreeding of this parent generation may have contributed to this situation. A sacrifice of chicks was made at the end of the brooding period to equalize group numbers to 25 females and 5 males for the next growth phase and to provide analytical material for tissue vitamin

Table 4. Effect of irradiation upon a chick starter ration as measured by tissue vitamin content analyses* - P_1 Generation.

Lot No.	Ration Treatment	Muscle		Liver	
		Thiamin γ/gm.	Riboflavin ug/gm.	Thiamin γ/gm.	Riboflavin ug/gm.
A	Irradiated	2.37	0.73	8.30	14.76
A_1	"	2.45	0.52	8.77	13.91
Mean		2.42	0.63	8.54	14.34
B	Non-irradiated	2.28	0.52	8.35	12.52
B_1	"	2.35	0.51	7.76	11.71
Mean		2.32	0.52	8.06	12.12

* Average of 15 birds per lot at age 7 weeks.

composition studies. Data for the growth period from 7 to 24 weeks are summarized and presented in Table 11. Growth response and feed efficiency results are somewhat variable with the control groups performing better than the irradiated groups. As before, these differences are probably not meaningful since analyses of variance on rate of gain and feed efficiency from week 9 to 12 (appendix Tables x - xii) indicated these differences not to be significant. Mortality, again, was a problem in this phase of the growth period resulting from a non-specific enteritis infection which developed after the birds were moved to their permanent quarters in the floor pens. This infection was treated with broad spectrum antibiotics and the birds responded rapidly. It is possible that the higher mortality of the D group resulted in poorer birds being culled out thus contributing to the superior growth response and feed efficieny of this group for the period under study.

As with the P_1 (parent) generation at approximately 28 weeks of age and following egg collection to produce the third generation, the replicate of each treatment group was discarded to provide adequate floor space and to conserve feed supplies. A summary of the body weight, egg production and feed efficiency data, for the remaining two groups during the laying period, is presented in Figures 4, 5 and 6. Differences between the two treatment groups in body weight for both males and females (Figure 4) and egg production (Figure 5) were not statistically significant and probably resulted primarily as a response to age and seasonal effects. An increasing trend toward more efficient feed conversion (Figure 6) by the control groups in the latter weeks of the trial may be a primary result of ration irradiation but no definite explanation can be given. Variability between birds and

mortality during the brooding and laying periods could have influenced this superior feed efficiency by the non-irradiated treatment group but the extent of their influence is difficult to estimate. Diagnostic gross examination on all birds which died during the laying period revealed no gross visible lesions or evidence which could suggest an effect due to ration irradiation.

The small differences in egg quality data as summarized in Table 5 may not be a primary result of ration treatment since an analysis of variance on Haugh Unit values (appendix Table xiv) indicated no significance due to ration treatment. A summary of the observations relative to reproductive performance in the replicate treatment groups is presented in Table 6. The differences are variable but tend to be small particularly with respect to hatchability of eggs set and appear not to be attributable to ration irradiation.

Analytical examinations of tissue for riboflavin and thiamin content from a randomly selected sample of the replicated groups are summarized and presented in Table 7. In all cases, with the exception of muscle riboflavin levels, the irradiated groups tended to have higher tissue vitamin levels but growth rate and feed consumption could be an influencing factor. Analyses of variance on the data (appendix Tables xv - xviii) indicate no significance due to ration treatment.

(c) Experiment 3 - F_2 Generation performance

The results of the brooding period in Experiment 3 are summarized and presented in Table 10. At 4 weeks of age, all groups were randomly

Table 5. Effect of irradiation upon a commercial layer ration as measured by egg quality criteria to age 34 weeks - F_1 Generation.

Lot No.	Period Weeks	Ration Treatment	Number Layers	Egg Quality* Size (gms.)	Sp.Gr.**	Haugh Units	Yolk Color
C	22-26	Irradiated	25	47.0	1.087	86.8	7
	26-30		25	47.5	1.090	65.3	6
	30-34		24	48.2	1.089	57.5	5.5
Mean				47.6	1.088	70.0	6.2
D_1	22-26	Non-irradiated	25	46.5	1.087	88.4	6.5
	26-30		25	47.4	1.085	69.5	5.9
	30-34		25	47.7	1.089	57.6	5.0
Mean				47.2	1.087	71.8	5.8

* All figures are an average of 10 eggs per group per period.

** Specific Gravity

Table 6. Effect of irradiation upon a commercial layer ration as measured by layer reproductive performance - F_1 Generation.

Lot No.	Ration Treatment	Egg Incubation Observations					
		Eggs Set	Infertile	Blood Rings	Dead Germs	Dead in Shell	% Hatch of Fertile Eggs Set
C	Irradiated	102	3	0	9	4	86.9
C_1	"	102	4	0	14	11	74.5
Mean %			3.4	0	11.3	7.4	80.7
D	Non-irradiated	102	4	0	11	11	77.6
D_1	"	102	4	0	7	8	84.7
Mean %			3.9	0	8.8	9.3	81.2
% Difference - Irrad. vs. Non-irrad.			(-)12.8	0	(+)28.4	(-)20.4	(-)0.6

Table 7. Effect of irradiation upon a chick starter ration as measured by tissue vitamin content analyses* - F_1 Generation.

Lot No.	Ration Treatment	Muscle		Liver	
		Thiamin γ/gm.	Riboflavin ug/gm.	Thiamin γ/gm.	Riboflavin ug/gm.
C	Irradiated	2.90	0.45	9.20	14.10
C_1	"	3.79	0.49	10.82	14.03
Mean		3.35	0.47	10.01	14.07
D	Non-irradiated	3.25	0.58	9.11	13.80
D_1	"	3.07	0.54	8.36	13.65
Mean		3.16	0.56	8.74	13.73

* Average of 5 birds per lot at age 7 weeks.

culled to 30 females and 7 males to equalize numbers according to sex and to provide more battery space. Generally, the non-irradiated groups performed slightly better than the irradiated groups in growth response and feed efficiency but the results tend to be variable and were not significant. Most of the mortality occurred, as would be expected, during the first few weeks of life due to small egg size with resultant weak chicks and hence unrelated to ration treatment. Results of the next growth phase to age 22 weeks are summarized and presented in Table 11. Growth response and feed efficiency for all groups was more uniform during this period of the trial and analyses of variance on rate of gain and feed efficiency, from week 5 to 11 (appendix Tables xix - xxii) indicated no significant differences which could be attributed to ration treatment. As may be noted from Table 11, mortality was not a problem during this phase of the trial.

All groups were placed on a layer ration at 22 weeks, rather than 24 weeks as done in the previous two generation studies, in an attempt to increase egg size at 28 weeks when eggs were collected for hatching. As in Experiments 1 and 2, the replicates of each treatment group were discarded at 28 weeks of age, following egg collection, to provide adequate housing space and to conserve feed supplies. A summary of body weight, egg production and feed efficiency data for the remaining two groups is presented in Figures 4, 5 and 6. The uniformity between treatment groups noted earlier in the trial continued through the laying period. Growth rate, egg production and feed efficiency performance of these two groups would not

appear to indicate any specific treatment effect other than age and seasonal effects. The low mortality experienced during the course of this trial signifies the lealthy response in treatment group performance.

The small differences in egg quality data as summarized in Table 8 do not appear significant since an analysis of variance on Haugh Unit values (appendix Table xxiii) indicated no significant differences due to ration treatment. The observations relative to reproductive performance for the replicate treatment groups, presented in Table 9, are quite variable. While hatchability of fertile eggs set was good in all groups, the irradiated groups had an inferior overall performance which may indicate an effect due to ration irradiation. Small egg size and age of the birds at collection, however, could have significantly influenced these variable results and more extensive studies would appear necessary to clarify this situation.

(d) Comparison of generation studies.

If the overall performances of the P_1(parent), F_1 and F_2 generations are examined, several comparisons may be drawn. Some comparative results have been combined and are presented in Tables 10 and 11 and Figures 4, 5 and 6. Generally, rate of gain, egg production and feed efficiency by the P_1 (parent) and F_2 Generation during the first 34 weeks of age were quite similar and superior to that of the F_1 Generation. This depression in performance of the F_1 Generation could most logically be attributed to the weak condition of the chicks upon hatching and the outbreak of non-specific enteritis during the growing period. Mortality for the

Table 8. Effect of irradiation upon a commercial layer ration as measured by egg quality criteria to age 34 weeks - F_2 Generation.

Lot No.	Period Weeks	Ration Treatment	Number Layers	Egg Quality* Size (gms.)	Sp.Gr**	Haugh Units	Yolk Color
G	18-20	Irradiated	30	40.0	1.092	73.3	6.3
	20-22		30	41.2	1.089	84.1	5.5
	22-26		20	44.2	1.085	83.0	5.9
	26-30		20	44.3	1.087	82.5	5.8
	30-34		20	46.5	1.086	79.9	5.3
Mean				43.2	1.088	80.6	5.8
G_1	18-20	Non-irradiated	29	41.3	1.090	69.4	6.8
	20-22		29	45.3	1.090	84.6	6.5
	22-26		20	45.9	1.087	88.1	6.4
	26-30		20	46.1	1.088	76.6	6.4
	30-34		19	47.3	1.086	77.8	6.4
Mean				45.2	1.088	79.3	6.5

* All figures are an average of 10 eggs per group per period.

**Specific Gravity

Table 9. Effect of irradiation upon a commercial layer ration as measured by layer reproductive performance - F_2 Generation.

Lot No.	Ration Treatment	Egg Incubation Observations					
		Eggs Set	Infertile	Blood Rings	Dead Germs	Dead in Shell	% Hatch of Fertile Eggs Set
E	Irradiated	100	19	5	14	1	75.3
G	"	100	9	4	13	2	79.1
Mean %			14.0	4.5	13.5	1.5	77.2
E_1	Non-irradiated	100	10	7	5	1	85.6
G_1	"	100	3	6	8	2	83.5
Mean %			6.5	6.5	6.5	1.5	84.6
% Difference - Irrad. vs. Non-irrad.			(+)115.4	(-)30.8	(+)107.7	0	(-)8.8

Table 10. Effect of irradiation upon a chick starter ration as measured by chick growth response and feed efficiency to age 7 weeks - P_1, F_1 and F_2 Generations.*

Lot No.	Initial Chick Nos.	Ration Treatment	Mean Body Wt.(gms.) Initial	Final	Gain	Average Feed Consumption/ Chick to 7 wks. (gms.)	Feed/ Gain Ratio	Mortality (%)
A	60	Irradiated	35.0	489.9	454.9	1171	2.57	3.3
A_1	60	(19% Basal Starter)	34.9	508.0	473.1	1171	2.48	3.3
Mean			35.0	499.0	464.0	1171	2.53	3.3
C	56		30.5	584.0	553.5	1496.8	2.70	15.9
C_1	56		28.8	605.7	576.9	1512.7	2.62	20.8
Mean			29.7	594.9	565.2	1504.8	2.66	18.4
E	86		29.2	582.1	552.9	1655.7	3.00	4.7
G	76		29.9	587.6	557.7	1776.5	3.19	3.9
Mean			29.6	584.9	555.3	1716.1	3.10	4.3
B	60	Non-irradiated	35.0	526.2	491.2	1180	2.40	8.5
B_1	60	(19% Basal Starter)	33.6	526.2	492.6	1383	2.81	3.3
Mean			34.3	526.2	491.9	1282	2.61	5.9
D	56		29.3	563.1	533.8	1471.4	2.76	12.3
D_1	56		31.0	581.6	550.6	1496.8	2.72	20.3
Mean			30.2	572.4	542.2	1484.1	2.74	16.3
E_1	73		29.8	593.0	563.2	1737.3	3.09	2.7
G_1	83		29.5	600.6	571.1	1742.7	3.05	3.6
Mean			29.7	596.8	567.2	1740.0	3.07	3.2

* P_1 Generation - Lot Nos. A, A_1, B, and B_1

F_1 Generation - Lot Nos. C, C_1, D, and D_1

F_2 Generation - Lot Nos. E, E_1, G, and G_1

Table 11. Effect of irradiation upon a chick grower ration as measured by chick growth response and feed efficiency from 7 to 24 weeks of age - P_1 & F_1 Generations and from 7 to 22 weeks of age - F_2 Generation. *

Lot No.	Final Bird Nos. ♀	♂	Ration Treatment	Mean Body Wt.(Kg.) Initial	Final	Gain	Average Feed Consumption/ Chick from 7 - 24 wks. (Kg.)	Feed/ Gain Ratio	Mortality (%)
A	23	10	Irradiated	0.49	1.95	1.46	11.68	8.00	2.94
A_1	22	10	(17% Basal Grower)	0.51	1.80	1.29	12.05	9.34	5.88
Mean				0.50	1.88	1.38	11.87	8.67	4.41
C	25	5		0.74	1.65	0.91	9.76	10.73	3.33
C_1	25	5		0.79	1.81	1.01	9.24	9.15	6.67
Mean				0.77	1.73	0.96	9.50	9.94	5.00
E	20	5		0.58	1.93	1.35	10.69	7.92	0
G	20	5		0.59	1.81	1.22	10.21	8.38	0
Mean				0.59	1.87	1.29	10.45	8.15	0
B	25	9	Non-irradiated	0.53	1.95	1.42	12.00	8.45	0
B_1	23	9	(17% Basal Grower)	0.52	1.91	1.39	12.00	8.63	5.88
Mean				0.53	1.93	1.41	12.00	8.54	2.94
D	25	5		0.74	1.96	1.21	10.40	8.60	16.67
D_1	25	5		0.80	1.96	1.16	10.57	9.11	0
Mean				0.77	1.96	1.19	10.49	8.86	8.33
E_1	20	5		0.59	1.84	1.25	10.26	8.22	0
G_1	20	5		0.60	1.87	1.27	10.29	8.11	0
Mean				0.60	1.86	1.26	10.28	8.17	0

* P_1 Generation - Lot Nos. A, A_1, B, and B_1

F_1 Generation - Lot Nos. C, C_1, D, and D_1

F_2 Generation - Lot Nos. E, E_1, G, and G_1

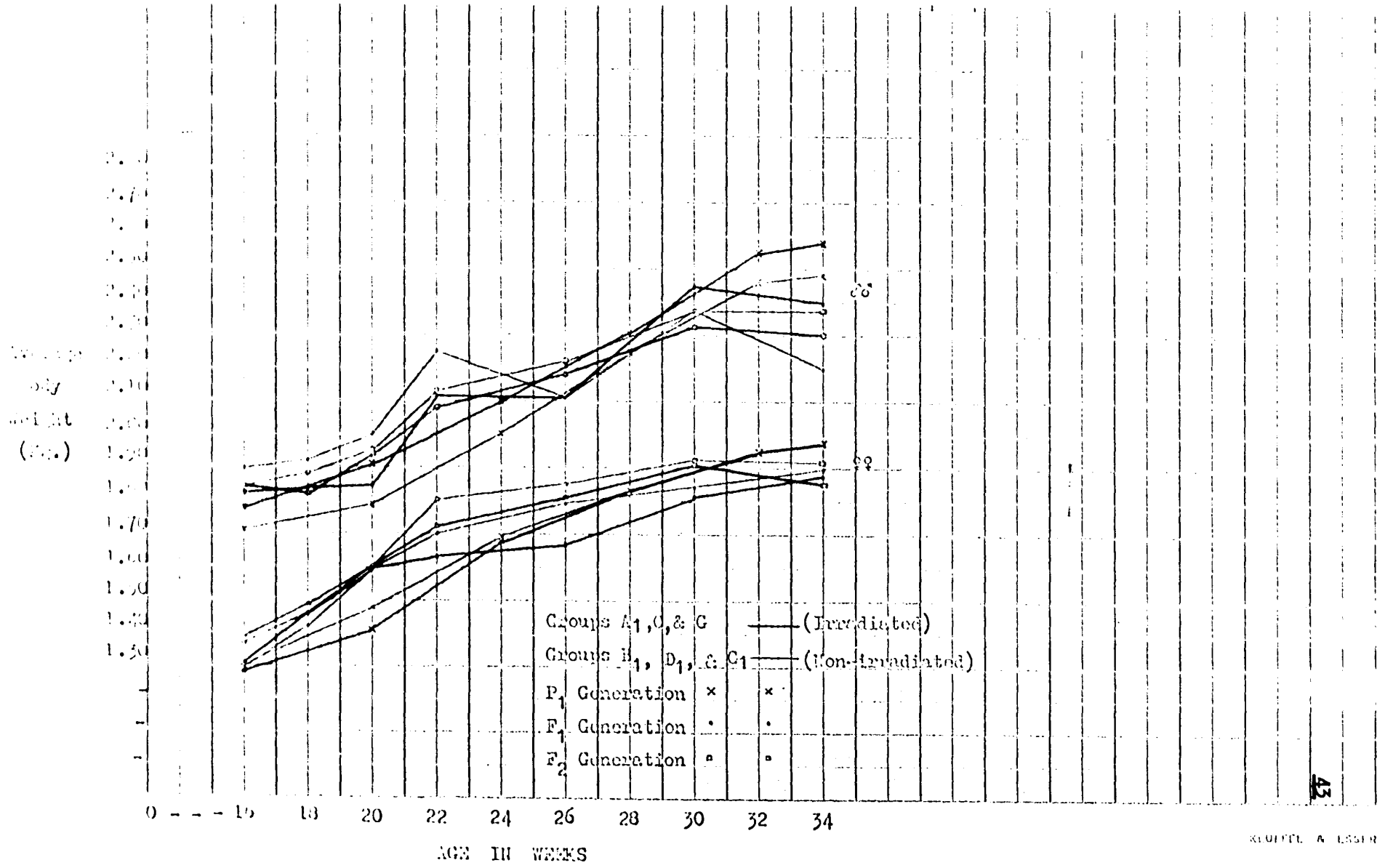

Figure 4. Effect of irradiation upon a commercial layer ration as measured by bird growth response to age 34 weeks - P_1, F_1 and F_2 Generations.

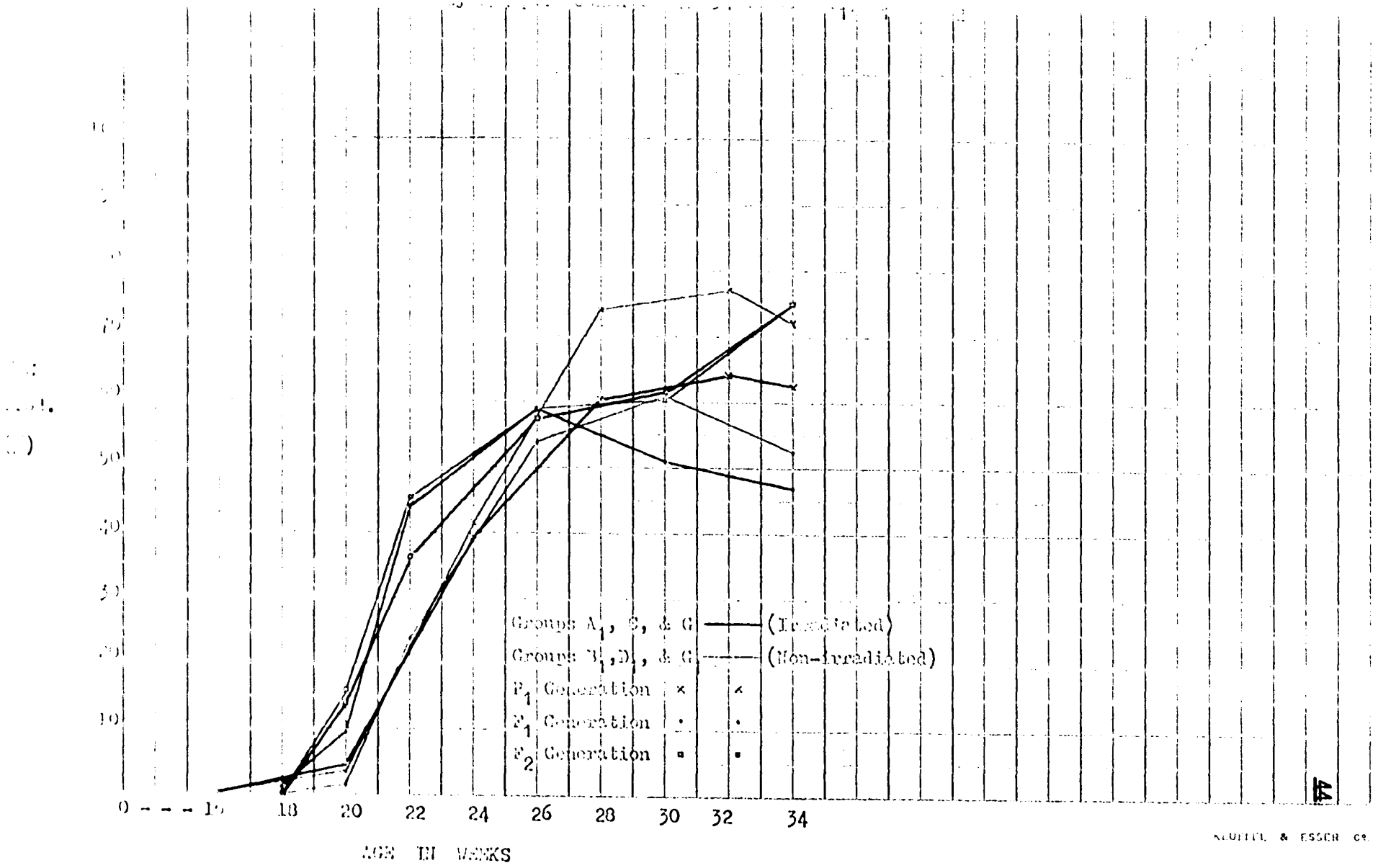

Figure 5. Effect of irradiation upon a commercial layer ration as measured by egg production to age 34 weeks - P_1, F_1, and F_2 Generations.

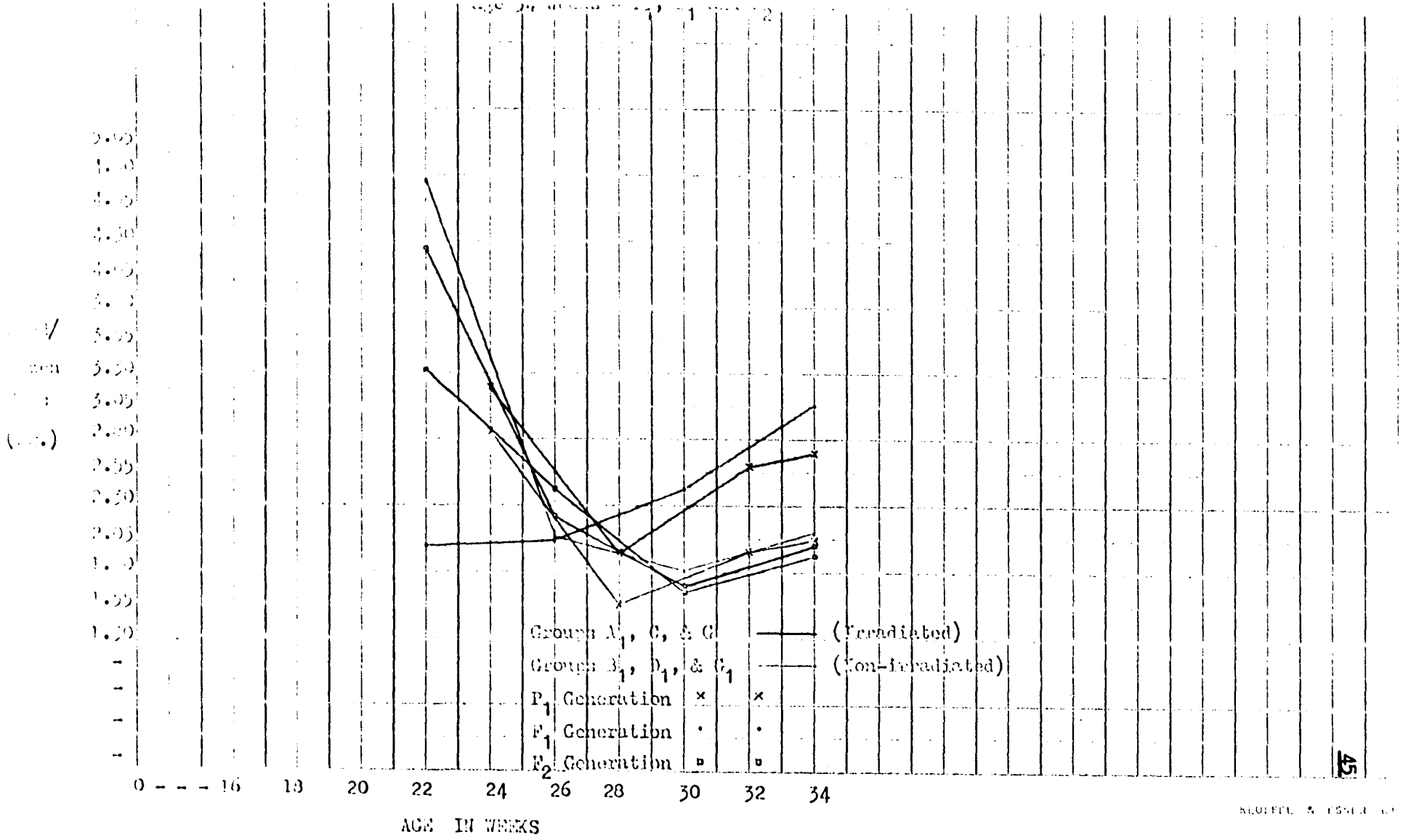

Figure 6. Effect of irradiation upon a commercial layer ration as measured by feed efficiency in egg production to age 34 weeks - P_1, F_1 and F_2 Generations.

period under comparison was lowest in the F_2 Generation and this may indicate a favourable effect due to inbreeding. Egg quality summaries, presented in previous tables, generally were similar in all generations.

The results of observations with respect to reproductive performance have been variable for all generations. As indicated in previous discussions, more extensive studies appear necessary in order to more fully evaluate any effect upon reproductive performance resulting from ration treatment. Tissue vitamin content values for the P_1 (parent) and F_1 Generations were similar and differences tended to be small.

The F_2 Generation produced the most uniform and consistent performance in most criteria studied to 34 weeks of age, with the exception of hatchability. This uniformity would seem to have resulted primarily from an inbreeding effect. No specific trends, which could be suggestive of a detrimental, cumulative effect resulting from the continuous feeding of irradiated rations to the three generations studied, were evident in the performance of this generation.

(e) Experiment 4 - Growth trial and tissue analysis

The growth trial results of Experiment 4 are summarized and presented in Table 12. Weekly data summaries with respect to rate of gain and feed efficiency and their analyses of variance are presented in appendix Tables xxiv - xxvii. The irradiated plus dilute treatment group (Lot 2) generally had the poorest performance but analyses of variance on rate of gain and feed efficiency indicated this performance not to be significantly different from that of the other treatment groups. This lack of apparent significance may suggest that the dilution factor used (25%) was

Table 12. Effect of irradiation and dilution* upon a chick starter ration as measured by chick growth response and feed efficiency to age 5 weeks - Experiment 4.

Lot No.	Initial Chick Nos.	Ration Treatment	Mean Body Wt.(gms.) Initial unsexed	Final unsexed	Gain	Average Feed Consumption/ Chick to 5 wks. (gms.)	Feed/ Gain Ratio	Mortality (%)
1	15	Irradiated	37.6	362.1	324.5	870.2	2.68	0
2	15	Irradiated + Dilute	37.4	346.6	309.2	851.4	2.75	0
Mean			37.5	354.4	316.9	860.8	2.72	
3	15	Non-irradiated	36.8	370.0	333.2	893.5	2.68	0
4	15	Non-irradiated + Dilute	38.6	372.1	333.5	888.6	2.67	0
Mean			37.7	371.1	333.4	891.1	2.68	

* Dilution factor of 25% utilizing a cereal protein mineral mix.

not sufficient to produce minimal vitamin requirement levels. Mortality, as may be noted from Table 12, was not a problem during this trial.

Tissue riboflavin and thiamin content data are summarized and presented in Table 13. Analyses of variance are presented in appendix Tables xxviii - xxxi. Ration treatment had no apparent significant influence on levels of muscle thiamin and liver riboflavin. There were significant differences ($P < 0.01$), however, in muscle riboflavin levels due to ration treatment. Application of the Duncan's Multiple Range Test revealed no significant effect from dilution of the irradiated ration but did indicate a significant response from dilution of the non-irradiated ration. Analyses of variance also indicated liver thiamin levels were significantly different ($P < 0.01$). Application of the Duncan's Multiple Range Test indicated that the liver thiamin level of the non-irradiated control group was significantly higher than for any other group and that dilution was effective to a certain extent in both the irradiated and non-irradiated rations. Since the liver thiamin level for the non-irradiated plus dilute group was not significantly different from that of either of the irradiated groups, it is difficult to interpret if any effect due to ration irradiation existed. The inconsistencies of these results, particularly with respect to significance of ration treatment, may have been influenced by small errors in experimental technique, although all analyses were run concurrently from day to day without interruption. With the exception of muscle thiamin levels in the non-irradiated plus dilute treatment group, there appears to be a trend toward response due to ration dilution. This trend, however, is not well established and more extensive studies using higher dilution levels would seem necessary to attain minimal or sub-optimal

Table 13. Effect of irradiation and dilution upon a chick starter ration as measured by tissue vitamin content analyses* - Experiment 4.

Lot No.	Ration Treatment	Muscle		Liver	
		Thiamin γ/gm.	Riboflavin ug/gm.	Thiamin γ/gm.	Riboflavin ug/gm.
1	Irradiated	4.48	0.92	11.78	18.91
2	Irradiated + Dilute	4.46	0.91	10.81	18.74
Mean		4.47	0.92	11.30	18.83
3	Non-irradiated	3.97	1.31	13.11	21.57
4	Non-irradiated + Dilute	4.07	1.09	11.36	19.93
Mean		4.02	1.20	12.24	20.75

* Average of 15 birds per lot at age 5 weeks.

vitamin requirement levels.

(f) Experiment 5 - Growth trial and tissue analysis

Data from the growth trial in Experiment 5 are summarized and presented in Table 14. Weekly data summaries, with respect to rate of gain and feed efficiency, and their analyses of variance are presented in appendix Tables xxxii - xxxv. Rate of gain by the 2.25 Mrad ration groups was significantly ($P < 0.01$) higher than that attained by the other treatment groups. Application of the Duncan's Multiple Range Test indicated that the rate of gain by the 3.5 Mrad ration group (Lot D) may be significantly higher than that of its replicate (Lot D_1) but the death of one chick in Lot D may have influenced this superior performance. It may be noted, at this time, that the chick which died showed no gross visible lesions or evidence upon diagnostic examination which could suggest an effect due to ration treatment. The performance of all groups with respect to feed efficiency was not significantly different. Since the performance of the 3.5 Mrad ration groups was similar, in most indices measured, to that of the control and 1 Mrad ration groups, it is difficult to interpret the performance of the 2.25 Mrad ration group as being due mainly to ration treatment. It appears that the significantly better rate of gain by the 2.25 Mrad groups was primarily a result of individual bird variability.

At the end of the growth trial, a randomly selected sample of the population was used for tissue riboflavin and thiamin content analyses. Data from these analyses are summarized and presented in Table 15. The results are quite variable with no consistent trends evident to suggest

Table 14. Effect of irradiation upon a chick starter ration as measured by chick growth response and feed efficiency to age 5 weeks - Experiment 5.

Lot No.	Initial Chick Nos.	Ration Treatment	Mean Body Wt.(gms.) Initial unsexed	Final unsexed	Gain	Average Feed Consumption/ Chick to 5 wks. (gms.)	Feed/ Gain Ratio	Mortality (%)
A	10	Non-irradiated	37.5	385.1	347.6	861.8	2.48	0
A_1	10	"	37.8	388.1	350.3	839.2	2.40	0
Mean			37.7	386.6	349.0	850.5	2.44	0
B	10	Irradiated (1Mrad)	39.2	389.1	349.9	861.8	2.46	0
B_1	10	"	39.0	374.4	335.4	816.5	2.43	0
Mean			39.1	381.8	342.7	839.2	2.45	0
C	10	Irradiated (2.25 Mrad)	38.5	422.1	383.6	895.9	2.34	0
C_1	10	"	37.0	415.0	378.0	907.2	2.40	0
Mean			37.8	418.6	380.8	901.6	2.37	0
D	10	Irradiated (3.5 Mrad)	37.1	392.2	355.1	940.0	2.65	10.0
D_1	10	"	37.4	366.7	329.3	771.1	2.34	0
Mean			37.3	379.5	342.2	855.6	2.50	5.0

Table 15. Effect of irradiation upon a chick starter ration as measured by tissue vitamin content analyses* - Experiment 5.

Lot No.	Ration Treatment	Muscle		Liver	
		Thiamin γ/gm.	Riboflavin ug/gm.	Thiamin γ/gm.	Riboflavin ug/gm.
A	Non-irradiated	1.50	0.57	5.20	11.51
A_1	"	2.01	0.37	7.73	11.17
Mean		1.76	0.47	6.47	11.34
B	Irradiated (1 Mrad)	1.60	0.61	6.97	14.36
B_1	"	1.81	0.44	7.75	12.41
Mean		1.71	0.53	7.36	13.39
C	Irradiated (2.25 Mrad)	1.71	0.53	6.53	12.08
C_1	"	1.92	0.51	7.22	11.85
Mean		1.82	0.52	6.88	11.97
D	Irradiated (3.5 Mrad)	1.52	0.36	6.80	11.28
D_1	"	1.93	0.43	7.72	9.69
Mean		1.73	0.40	7.26	10.49

* Average of 5 birds per lot at age 5 - 6 weeks.

a possible effect due to ration treatment and analyses of variance (appendix Tables xxxvi - xxxix) indicate no significance due to ration treatment.

C. TBA TEST - EXPERIMENT 6

The results of Experiment 6 are summarized and presented in Table 16. The analysis of variance of this data is presented in appendix Table xl. A consistent trend toward reduced fat stability upon irradiation of the poultry rations was observed in all trials. Analysis of variance of the data, however, indicated no significant differences due to ration treatment. It would appear therefore that an irradiation dose higher than 3.5 Mrad would be necessary before fat stability could be significantly altered. The differences between trials for each ration treatment are variable but the analysis of variance indicated none were significant. Possibly the random selection of samples from the storage stacks in each trial could account for most of this variability. Length of storage of the rations may have influenced the results but the period between trials was not more than two weeks. Small differences in experimental technique between trials could also have influenced the results. An additional source of variability may have arisen from slight variation in the quality of the fat sources for the starter and grower rations since each ration was mixed and prepared separately during manufacture.

Table 16. Effect of irradiation upon fat stability in chick starter and grower rations as measured by the TBA Test - Experiment 6.

Ration	Ration Treatment	Mg. of M.A./gm. Fat*			
		Trial 1	Trial 2	Trial 3	Mean
Starter	Non-irradiated	0.312	0.273	0.390	0.325
	Irradiated (1 Mrad)	0.390	0.332	0.390	0.371
	Irradiated (3.5 Mrad)	0.488	0.449	0.429	0.455
Grower	Non-irradiated	0.293	0.273	0.410	0.325
	Irradiated (1 Mrad)	0.332	0.488	0.449	0.423

* Average of 5 randomly selected samples per treatment per trial.

VI. SUMMARY AND CONCLUSIONS

The purpose of these studies was to provide further information upon the feasibility of radiation disinfection of poultry rations and particularly to study the effect of gamma irradiation upon nutrient stability in poultry rations. Six experiments were conducted. Experiments 1, 2 and 3 involved three concurrent generation studies with the P_1(parent), F_1 and F_2 Generations, respectively, being fed irradiated and non-irradiated rations to 84 weeks in the case of Experiment 1 and to 34 weeks in each of the other two experiments. In Experiment 4, a short-term growth trial was established to study the effect of diluting the vitamin content of irradiated rations. Experiment 5, another short-term growth trial, was undertaken to determine the effect of irradiating poultry rations at different dose levels (i.e. 1.0, 2.25 and 3.5 Mrad). Experiment 6 involved utilization of the TBA Test to secure an indication of possible ration fat degradation upon irradiation.

No specific trends developed in such criteria as growth response, feed efficiency, mortality, egg production, and egg quality during the course of the generation studies which could be attributed to ration irradiation. The reproductive performance of all generations was quite variable and more extensive studies are felt necessary before definite conclusions can be drawn. Levels of riboflavin and thiamin in muscle and liver tissue of birds in Experiments 1 and 2 were not significantly affected by ration treatment.

Ration irradiation and dilution had no significant effect on growth response and feed efficiency of birds involved in Experiment 4. The

inconsistencies of results with respect to thiamin and riboflavin tissue levels, in this experiment, suggest further studies utilizing higher dilution levels are necessary in order to establish the effect of ration irradiation on tissue storage of these vitamins.

The superior growth response of the 2.25 Mrad ration treatment group in Experiment 5 was felt to be mainly a result of individual bird variability rather than ration treatment since only ten birds were used in each replicate and the response of the 3.5 Mrad treatment group did not differ significantly from that of the control group. Bird tissue levels of thiamin and riboflavin were not significantly altered by the higher irradiation dose levels used in this experiment.

The results of Experiment 6 indicated that fat stability in poultry rations was reduced with increasing irradiation dose levels but that no significant changes occur at the 1.0 Mrad dose level being considered for ration disinfection.

The results of these experiments suggest that poultry rations irradiated at 1.0 Mrad are not significantly altered in nutrient content and that poultry should respond normally when fed these rations. It is felt, however, that the possible effect of ration irradiation on reproductive performance warrants more intensive investigation particularly after the birds reach full maturity. It is also recognised that tissue levels of thiamin and riboflavin analyzed in these studies were quite variable and that reduction of this variability possibly would have been obtained through correlation of these values with total liver and breast muscle weights of individual birds sampled.

LITERATURE CITED

Alexander, H. D., E. J. Day, H. E. Sauberlich and W. D. Salmon. (1956). Radiation effect on water soluble vitamins in raw beef. Fed. Proc. 15: 921.

Bernheim, F., M. L. C. Bernheim and K. M. Wilbur. (1948). The reaction between thiobarbituric acid and the oxidation products of certain lipids. J. Biol. Chem. 174: 257.

Biggs, D. A. and L. R. Bryant. (1953). The thiobarbituric acid test for butterfat oxidation. Can. J. Tech. 31: 138.

Brin, M., A. S. Ostashever, M. Tai and H. Kalinsky. (1961). Effects of feeding X-irradiated pork to rats on their thiamine nutrition as reflected in the activity of erythrocyte transketolase. J. Nutr. 75: 29.

Brooke, R. O., E. M. Ravesi, D. F. Gadbois and M. A. Steinberg. (1966). Preservation of fresh unfrozen fishery products by low-level radiation. 5. The effects of radiation pasteurization on amino acids and vitamins in haddock fillets. Food Tech. 20: 99.

Bryan, F. L., J. C. Ayres and A. A. Kraft. (1968). Contributory sources of Salmonellae on turkey products. Amer. J. Epidemiology. 87:578.

Burns, C. H., L. E. Brownell and H. C. Eckstein. (1956). Wholesomness of a gamma-irradiated diet fed to chickens. Fed. Proc. 15:910.

Cain, R. F., E. C. Bubl and A. W. Anderson. (1956). The effect of intermittent radiations and concomitant increase in temperature during radiation on the acceptability of ground beef. Food Tech. 10: 537.

Caldwell, E. F. and B. Grogy. (1955). Application of the thiobarbituric acid test to cereal and baked food. Food Tech. 9: 185.

Coates, M. E., R. Fuller, G. F. Harrison, M. Lev and S. F. Suffolk. (1963). A comparison of the growth of chicks in the Gustafson germfree apparatus in a conventional environment with and without dietary supplements of penicillin. Brit. J. Nutr. 17: 141.

Cornwell, P. B. (1959). Effect of γ radiation on the taste and manufacturing properties of soft wheat. J. Sci. Fd. Agric. 10: 409.

Dox, A. W. and G. P. Plaisance. (1916). The condensation of thiobarbituric acid with aromatic aldehydes. J. Am. Chem. Soc. 38: 2164.

Ferrando, R., J. Pantaléon and D. Fremageot. (1968). Comparative study of conventional sterilization and irradiation of rat food. Ann. Nutr. Aliment 22: 25. Cit. Nutr. Abs. and Rev. 38: 1245.

Greene, B. E. and B. M. Watts. (1966). Lipid oxidation in irradiated cooked beef. Food Tech. 20: 111.

Groninger, H. S. and A. L. Tappel. (1957). The destruction of thiamine in meats and in aqueous solution by gamma radiation. Food. Res. 22: 519.

Idziak, E. S. and K. Incze. (1968). Radiation treatment of foods. I. Radurization of fresh eviscerated poultry. Appl. Microbiol, 16: 1061.

International Atomic Energy Agency. (1963). Radiation Control of Salmonella in Food and Feed Products. Technical Report Series No.22., Vienna.

Johnson, B. C., M. S. Mameesh, V. C. Metta and P. B. Rama Roa. (1960). Vitamin K nutrition and irradiation sterilization. Fed. Proc. 19: 1038.

Kennedy, T. S. (1965). Studies on the nutritional value of foods treated with γ - radiation. I. Effects on some B-complex vitamins in egg and wheat. J. Sci. Fd. Agric. 16: 8.

Kennedy, T. S. (1965). Studies on the nutritional value of foods treated with γ-radiation. II. Effects on the protein in some animal feeds, egg and wheat. J. Sci. Fd. Agric. 16: 433.

Kertesz, Z. I., E. R. Schulz, G. Fox and M. Gibson. (1959). Effects of ionizing radiations on plant tissues. 4. Some effects of gamma radiation on starch and starch fractions. Food Res. 24: 609.

Kwon, T. W., D. B. Menzel and H. S. Alcott. (1965). Reactivity of malonaldehyde with food constituents. J. Fd. Sci. 30: 808.

Lees, D. H. (1967). Off-flavour development in frozen green beans. M. Sc. Thesis. McGill University, Montreal.

Leistner, L., J. Johantges, R. H. Deibel and C. F. Niven. (1961). The occurrence and significance of Salmonellae in meat animals and animal by-product feeds. Amer. Meat Inst. Foundations Res. Conf. Proc. 13: 9.

Ley, F. J., B. M. Freeman and B. C. Hobbs. (1963). The use of gamma radiation for the elimination of Salmonellae from various foods. J. Hyg., Camb. 61: 515.

Metta, V. C. and B. C. Johnson. (1959). Biological value of gamma-irradiated corn gluten and wheat gluten. J. Agric. Fd. Chem. 7: 131.

Metta, V. C., M. S. Mameesh and B. C. Johnson. (1959). Vitamin K deficiency in rats induced by the feeding of irradiated beef. J. Nutr. 69: 18.

Ministry of Health. Committee on Medical and Nutritional Aspects of Food Policy. (1964). Report of the Working Party on Irradiation of Food. Her Majesty's Stationery Office, London.

Morgan, B. H. (1958). Radiation chemistry of foods. Proc. 2nd. U.N. Int. Conf. Peaceful Uses of Atomic Energy. Geneva. 27: 423.

Mossel, D. A. A., M. van Schothorst and E. H. Kampelmacher. (1967). Comparative study on decontamination of mixed feeds by radicidation and pelletisation. J. Sci. Fd. Agric. 18: 362.

Patton, S. and G. W. Kurtz. (1951). 2-thiobarbituric acid as a reagent for detecting milk oxidation. J. Dairy Sci. 34: 669.

Read, M. S. (1960). A summary of wholesomeness of gamma-irradiated foods. Fed. Proc. 19: 1055.

Read, M. S. (1960a). The effects of ionizing radiations on the nutritive value of foods. Proceedings of the International Conference on the Preservation of Foods by Ionizing Radiations. July,1959. pp. 138-152. Washington, D. C. United States Atomic Energy Commission Technical Information.

Richardson, L. R., P. Woodworth and S. Coleman. (1956). Effect of ionizing radiations on vitamin K. Fed. Proc. 15: 924.

Richardson, L. R., J. L. Martin and S. Hart. (1958). The activity of certain water-soluble vitamins after exposure to gamma radiations in dry mixture and in solution. J. Nutr. 65: 409.

Ritchey, S. J. and L. R. Richardson. (1960). The effect of irradiated vegetable oils and animal fatty tissue and storage of the diet on growth and mortality in chicks. Poultry Sci. 39: 404.

Saslow, L. D. and V. S. Warandekar. (1965). Behaviour of unsaturated fatty acids in the thiobarbituric acid test after radiolysis. Rad. Res. 24: 375. cf. Chem. Abs. 62: 13492e.

Schrieber, M. and E. S. Nasset. (1959). Digestion of irradiated fat *in vivo*. J. Appl. Physiol. 14: 639.

Sribney, M., U. J. Lewis and B. S. Schweigert. (1955). Effect of irradiation on meat fats. J. Agr. Fd. Chem. 3: 958.

Sheffner, A. L., R. Adachi and H. Spector. (1957). The effect of radiation processing upon the *in vitro* digestibility and nutritional qualities of proteins. Food Res. 22: 445.

Simnhuber, R. O. and T. C. Yu. (1958). 2-thiobarbituric acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. Food Tech. 12: 9.

Smith, R. B. (1966). Off-flavour development in frozen cauliflower. M. Sc. Thesis. McGill University, Montreal.

Steel, R. G. D. and J. H. Torrie (1960). Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., Toronto.

Tarladgis, B. G. and B. M. Watts. (1960). Malonaldehyde production during the controlled oxidation of pure, unsaturated fatty acids. J. Am. Oil Chem. Soc. 37: 403.

Tarladgis, B. G., B. W. Watts, M. T. Younathan and L. R. Dugan. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44.

Tarladgis, B. G., A. M. Pearson and L. R. Dugan. (1964). Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II. Formation of the thiobarbituric acid-malonaldehyde complex without acid-heat treatment. J. Sci. Fd. Agric. 15: 602.

Thornley, M. J. (1964). The Salmonellosis Problem and Prospects for the Irradiation Treatment of Foods. Paper presented at the International Conference on Radiation Preservation of Foods. National Academy of Sciences et al., Boston.

Tipples, K. H. and F. W. Norris. (1965). Some effects of high levels of gamma irradiation on the lipids of wheat. Cereal Chem. 42: 437.

U. S. Atomic Energy Commission, Division of Technical Information. (1966). Nuclear terms - a brief glossary. Oak Ridge, Tennessee.

U. S. Department of Commerce, Business and Defence Services Administration. (1965). Current status and commercial prospects for radiation preservation of food. A report for Division of Isotopes Development. U. S. Atomic Energy Commission, Washington, D.C.

Wilbur, K. M., F. Bernheim and O. W. Shapiro. (1949). The thiobarbituric acid reagent as a test for the oxidation of unsaturated fatty acids by various agents. Arch. Biochem. 24: 305.

Wilson, G. M. (1959). The treatment of meats with ionising radiations. 2. Observations on the destruction of thiamine. J. Sci. Fd. Agric. 10: 295.

Young, F. G. (1964). The production and properties of gamma-radiation and of high speed electrons and their use in the treatment of food. Ministry of Health. Committee on Medical and Nutritional Aspects of Food Policy. Report of the Working Party on Irradiation of Food. Her Majesty's Stationery Office, London.

APPENDIX

APPENDIX I

THIAMIN DETERMINATION

1. Pipette 1 ml. aliquots into each of two 25 ml. cylinders.
2. Make up to 1.5 ml. with distilled water.
3. To each add 2 ml. absolute methanol and 1 ml. 20% NaOH, mix.
4. Add immediately to 1 cylinder only, 2 drops of a 2% solution of $K_3Fe(Cn)_6$.
5. Add 1 ml. 30% H_2O_2 to blank and unknown.
6. Let stand 30 seconds.
7. Add 10 ml. of water saturated isobutanol.
8. Shake vigorously 4 minutes then let stand in dark 2 minutes.
9. Pipette 5 ml. of supernatant isobutanol extract into cuvette.
10. Add 2 ml. of 95% ethanol and mix thoroughly.
11. Repeat procedure including blank, using 1 ml. of standard thiamin solution.
12. Read fluorescence of the 4 isobutanol-ethanol solutions.

APPENDIX II

RIBOFLAVIN DETERMINATION

1. Pipette 25 ml. of extract into 50 ml. volumetric.
2. Add 0.5 ml. soldium hydrosulfite solution (2.5%).
3. Add 1.25 ml. stannous chloride working solution (.0008 gm/ml.).
4. Dilute to volume, mix and allow to stand 10 minutes.
5. Pour into unstoppered 250 ml. Erlenmeyer flask and shake 30 minutes.
6. Pipette 15 ml. into cuvette and take reading A.
7. Add 0.1 ml. of riboflavin working solution (15 ug./ml.)
8. Mix thoroughly.
9. Take reading B.
10. Add 2 drops sodium hydrosulfite solution. Take reading C.
11. Check for completeness of riboflavin reduction.

APPENDIX III

LIPID EXTRACTION PROCEDURE

1. Add 68 mls. water to 100 gms feed (assuming 12% moisture).
2. Homogenize in Waring Blendor for 2 minutes with 100 ml. chloroform and 200 mls. methanol.
3. Add 100 mls. chloroform and blend 30 seconds.
4. Add 100 mls. distilled water and blend 30 seconds.
5. Filter through Whatman #1 filter paper on a Coors #3 Buchner funnel with slight suction.
6. Transfer to 500 ml. graduated cylinder and allow to separate.
7. Remove the alcoholic-water layer by aspiration.
8. Evaporate chloroform layer under a stream of nitrogen in a water bath at 40 - 50 ^{o}C
9. Decant 2 - 3 times with petroleum ether to remove protein.
10. Pool extracts and evaporate under nitrogen in a water bath.
11. Stopper flask and store in a freezer at 0^{o}C until tested.

APPENDIX IV

ANALYSIS OF VARIANCE : BASIC FORMS

1. Tissue Vitamin Content and TBA Test

Correction Factor: _____

Total S.S.:_____

Treatment S.S.: _____

Replicate S.S.: _____

Plot Total S.S.: _____

Experimental Error S.S.: _____

Sampling Error S.S.: _____

Source of Variation	D.F.	S.S.	M.S.	F.	P.05	P.01
Treatment						
Replicate						
Experimental Error						
Sampling Error						
Total						

2. Rate of Gain and Feed Efficiency

Correction Factor: _____

Total S.S.: _____

Treatment S.S.: _____

Replicate S.S.: _____

Weeks S. S.: _____

Treatment x Weeks S. S.: _____

Error S.S.: _____

Source of Variation	D.F.	S.S.	M.S.	F.	P.05	P.01
Treatment						
Replicate						
Weeks						
Treatment x Weeks						
Error						
Total						

Appendix Table (i) - Average Rate of Gain* (gms.)
(Summary Experiment 1 - P_1 Generation)

Lot No.	Week 8	Week 10	Week 12	Week 14
A (Irrad.)	237.47	302.85	96.06	115.46
A_1 (Irrad.)	281.50	226.80	144.09	98.73
B (Non-irrad.)	216.13	217.46	112.07	198.78
B_1 (Non-irrad.)	192.11	212.13	129.41	133.41

* Average of 24 ♀♀ and 10 ♂♂ per lot.

ANALYSIS OF VARIANCE

Appendix Table (ii) - Average Rate of Gain.
(Summary Experiment 1 - P_1 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	522.81	522.81	0.50	5.99	12.25
Replicate	1	381.22	381.22	0.36	5.99	12.25
Weeks	3	46709.76	15569.92	14.80	4.35	8.45
Treatment x Weeks	3	8526.92	2842.31	2.70	4.35	8.45
Error	7	7362.93	1051.85			
Total	15	63503.64				

Appendix Table (iii) - Average Feed/Gain Ratio*
(Summary Experiment 1 - P_1 Generation)

Lot No.	Week 8	Week 10	Week 12	Week 14
A (Irrad.)	4.33	5.33	13.75	11.01
A_1 (Irrad.)	3.93	6.65	9.77	11.55
B (Non-irrad.)	5.49	6.69	11.07	6.30
B_1 (Non-irrad.)	6.88	6.76	8.35	8.80

* Average of 24 ♀♀ and 10♂♂ per lot.

ANALYSIS OF VARIANCE

Appendix Table (iv) - Average Feed/Gain Ratio
(Summary Experiment 1 - P_1 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	2.23	2.23	0.93	5.99	12.25
Replicate	1	0.10	0.10	0.04	5.99	12.25
Weeks	3	80.93	26.98	11.29	4.35	8.45
Treatment x Weeks	3	20.66	6.89	2.88	4.35	8.45
Error	7	16.70	2.39			
Total	15	120.62				

ANALYSIS OF VARIANCE

Appendix Table (v) - Egg Quality - Haugh Unit Values.
(Summary Experiment 1 - P_1 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	51.52	51.52	0.006	4.75	9.33
Weeks	12	9423.38	785.28	0.09	2.69	4.16
Error	12	103829.38	8652.45			
Total	25					

ANALYSIS OF VARIANCE

Appendix Table (vi) - Muscle Thiamin Levels
(Summary Experiment 1 - P_1 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Replicate	1	0.08	0.08	0.73	4.00	7.08
Treatment	1	0.15	0.15	1.38	4.00	7.08
Experimental Error	1	0.00	0.00			
Sampling Error	56	6.08	0.109			
Total	59	6.31				

ANALYSIS OF VARIANCE

Appendix Table (vii) - Muscle Riboflavin Levels
(Summary Experiment 1 - P_1 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Replicate	1	0.16	0.16	1.07	161.4	4052.0
Treatment	1	0.18	0.18	1.20	161.4	4052.0
Experimental Error	1	0.15	0.15			
Sampling Error	56	0.86	0.02			
Total	59	1.35				

ANALYSIS OF VARIANCE

Appendix Table (viii) - Liver Thiamin Levels
(Summary Experiment 1 - P_1 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	3.49	3.49	0.05	161.4	4052.0
Replicate	1	0.06	0.06	0.01	161.4	4052.0
Experimental Error	1	4.25	4.25			
Sampling Error	56	38.65	0.69			
Total	59	46.45				

ANALYSIS OF VARIANCE

Appendix Table (ix) - Liver Riboflavin Levels
(Summary Experiment 1 - P_1 Generation

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	74.02	74.02	22.09	4.00	7.08
Replicate	1	10.29	10.29	3.07	4.00	7.08
Experimental Error	1	0.00	0.00			
Sampling Error	56	187.71	3.35			
Total	59	272.03				

Appendix Table (x) - Average Rate of Gain* (gms.)
(Summary Experiment 2 - F_1 Generation)

Lot No.	Week 9	Week 10	Week 11	Week 12
C (Irrad.)	38.56	134.57	104.33	98.28
C_1 (Irrad.)	44.60	164.05	42.34	74.84
D (Non-irrad.)	92.99	164.05	16.63	62.74
D_1 (Non-irrad.)	46.12	179.17	68.04	92.23

* Average of 25 ♀♀ and 5 ♂♂ per lot

ANALYSIS OF VARIANCE

Appendix Table (xi) - Average Rate of Gain
(Summary Experiment 2 - F_1 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	26.02	26.02	0.03	5.59	12.25
Replicate	1	0.04	0.04	0.0001	5.59	12.25
Weeks	3	28976.82	9658.94	12.04	4.35	8.45
Treatment x Weeks	3	2297.23	765.74	0.95	4.35	8.45
Error	7	5617.87	802.55			
Total	15	36917.96				

Appendix Table (xii) - Average Feed/Gain Ratio*
(Summary Experiment 2 - F_1 Generation)

Lot No.	Week 9	Week 10	Week 11	Week 12
C (Irrad.)	12.16	4.21	5.22	5.62
C_1 (Irrad.)	11.53	3.13	12.14	3.06
D (Non-irrad.)	4.47	2.72	26.84	9.28
D_1 (Non-irrad.)	12.46	3.33	8.22	7.21

* Average of 25 ♀♀ and 5 ♂♂ per lot.

ANALYSIS OF VARIANCE

Appendix Table (xiii) - Average Feed/Gain Ratio
(Summary Experiment 2 - F_1 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	19.05	19.05	0.58	5.59	12.25
Replicate	1	5.57	5.57	0.17	5.59	12.25
Weeks	3	220.25	73.42	2.23	4.35	8.45
Treatment x Weeks	3	86.37	28.79	0.88	4.35	8.45
Error	7	230.04	32.86			
Total	15	561.27				

ANALYSIS OF VARIANCE

Appendix Table (xiv) - Egg Quality - Haugh Unit Values
(Summary Experiment 2 - F_1 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	5.79	5.79	0.0004	18.51	98.50
Weeks	2	938.71	469.36	0.03	19.00	99.00
Error	2	30122.65	15061.33			
Total	5					

ANALYSIS OF VARIANCE

Appendix Table (xv) - Muscle Thiamin Levels
(Summary Experiment 2 - F_1 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	0.17	0.17	0.12	161.4	4052.0
Replicate	1	0.60	0.60	0.41	161.4	4052.0
Experimental Error	1	1.45	1.45			
Sampling Error	16	2.11	0.13			
Total	19	4.33				

ANALYSIS OF VARIANCE

Appendix Table (xvi) - Muscle Riboflavin Levels
(Summary Experiment 2 - F_1 Generation).

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	0.04	0.04	4.0	161.4	4052.0
Replicate	1	0.00	0.00	0	161.4	4052.0
Experimental Error	1	0.01	0.01			
Sampling Error	16	0.10	0.01			
Total	19	0.14				

ANALYSIS OF VARIANCE

Appendix Table (xvii) - Liver Thiamin Levels
(Summary Experiment 2 - F_1 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	8.11	8.11	1.15	161.4	4052.0
Replicate	1	0.95	0.95	0.13	161.4	4052.0
Experimental Error	1	7.08	7.08			
Sampling Error	16	12.01	0.75			
Total	19	28.15				

ANALYSIS OF VARIANCE

Appendix Table (xviii) - Liver Riboflavin Levels
(Summary Experiment 2 - F_1 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	0.58	0.58	58.0	161.4	4052.0
Replicate	1	0.06	0.06	6.0	161.4	4052.0
Experimental Error	1	0.01	0.01			
Sampling Error	16	29.58	1.85			
Total	19	30.22				

Appendix Table (xix) - Average Rate of Gain* (gms.)
(Summary Experiment 3 - F_2 - Generation)

Lot No.	Week 5	Week 6	Week 7	Week 9	Week 11
E (Irrad)	74.95	108.08	94.95	235.68	197.84
E_1 (Non-irrad.)	68.16	120.51	93.16	212.16	202.97
G (Irrad.)	64.41	126.41	95.19	242.43	201.35
G_1 (Non-irrad.)	59.41	129.30	79.57	217.32	219.97

* Average of 30 ♀♀ and 7 ♂♂ per lot.

ANALYSIS OF VARIANCE

Appendix Table (xx) - Average Rate of Gain (gms.)
(Summary Experiment 3 - F_2 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	75.12	75.12	1.24	5.12	10.56
Replicate	1	36.18	36.18	0.60	5.12	10.56
Weeks	4	79906.91	19976.73	330.91	3.63	6.42
Treatment x Weeks	4	826.35	206.59	3.42	3.63	6.42
Error	9	543.37	60.37			
Total	19	81387.93				

Appendix Table (xxi) - Average Feed/Gain Ratio*
(Summary Experiment 3 - F_2 Generation)

Lot No.	Week 5	Week 6	Week 7	Week 9	Week 11
E (Irrad.)	3.60	2.95	3.49	4.01	6.57
E_1 (Non-irrad.)	3.60	2.65	3.82	4.05	5.68
G (Irrad.)	4.00	2.62	5.28	3.94	5.78
G_1 (Non-irrad.)	4.12	2.53	4.91	4.12	5.16

* Average of 30 ♀♀ and 7 ♂♂ per lot.

ANALYSIS OF VARIANCE

Appendix Table (xxii) - Average Feed/Gain Ratio
(Summary Experiment 3 - F_2 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	0.12	0.12	0.40	5.12	10.56
Replicate	1	0.21	0.21	0.70	5.12	10.56
Weeks	4	20.07	5.02	16.73	3.63	6.42
Treatment x Weeks	4	0.52	0.13	0.43	3.63	6.42
Error	9	2.71	0.30			
Total	19	23.63				

ANALYSIS OF VARIANCE

Appendix Table (xxiii) - Egg Quality - Haugh Unit Values
(Summary Experiment 3 - F_2 Generation)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	1	3.97	3.97	0.0003	7.71	21.20
Weeks	4	252.12	63.03	0.004	6.39	15.98
Error	4	63924.40	15981.10			
Total	9					

Appendix Table (xxiv) - Average Rate of Gain* (gms.)
(Summary Experiment 4)

Lot No.	Week 1	Week 2	Week 3	Week 4	Week 5
1 (Irrad.)	38.03	59.07	67.80	72.40	84.20
2 (Irrad. + Dil.)	34.67	58.07	73.53	68.33	74.20
3 (Non-irrad.)	39.72	62.27	68.87	75.80	83.80
4 (Non-irrad. + Dil.)	37.85	62.13	67.33	72.53	90.80

* Average of 15 birds per lot unsexed.

ANALYSIS OF VARIANCE

Appendix Table (xxv) - Average Rate of Gain
(Summary Experiment 4)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	3	63.56	21.19	1.63	3.49	5.95
Weeks	4	4711.86	1177.97	90.75	3.26	5.41
Error	12	155.81	12.98			
Total	19	4931.23				

Appendix Table (xxvi) - Average Feed/Gain Ratio*
(Summary Experiment 4)

Lot No.	Week 1	Week 2	Week 3	Week 4	Week 5
1 (Irrad.)	1.65	2.04	2.12	3.36	3.24
2 (Irrad. + Dil.)	1.75	1.48	2.32	3.24	3.79
3 (Non-irrad.)	1.60	1.90	2.30	3.64	3.33
4 (Non-irrad. + Dil.)	1.68	1.94	2.41	3.07	3.45

* Average of 15 birds per lot unsexed.

ANALYSIS OF VARIANCE

Appendix Table (xxvii) - Average Feed/Gain Ratio
(Summary Experiment 4)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	3	0.01	0.003	0.063	3.49	5.95
Weeks	4	11.04	2.76	57.50	3.26	5.41
Error	12	0.57	0.048			
Total	19	11.62				

ANALYSIS OF VARIANCE

Appendix Table (xxviii) - Muscle Riboflavin Levels
(Summary Experiment 4)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	3	1.20	0.40	13.33	2.76	4.13
Error	56	1.71	0.03			
Total	59					

Duncan's Multiple Range Test

Treatment	Non-irrad.	Non-irrad. + Dilute	Irrad.	Irrad. + Dilute
Muscle Riboflavin ug/gm	1.31	1.09	0.92	0.91

ANALYSIS OF VARIANCE

Appendix Table (xxix) - Liver Riboflavin Levels
(Summary Experiment 4)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	3	76.00	25.33	1.91	2.76	4.13
Error	56	742.20	13.25			
Total	59					

ANALYSIS OF VARIANCE

Appendix Table (xxx) - Liver Thiamin Levels
(Summary Experiment 4)

Source of Variation	DF	SS	MS	F	P.05	P.05
Treatment	3	42.77	14.26	9.70	2.76	4.13
Error	56	82.56	1.47			
Total	59					

Duncan's Multiple Range Test

Treatment	Non-irrad.	Irrad.	Non-irrad. + Dilute	Irrad. + Dilute
Liver Thiamin γ/gm.	13.11	11.78	11.36	10.81

ANALYSIS OF VARIANCE

Appendix Table (xxxi) - Muscle Thiamin Levels
(Summary Experiment 4)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	3	3.16	1.05	1.59	2.76	4.13
Error	56	37.17	0.66			
Total	59					

Appendix Table (xxxii) - Average Rate of Gain* (gms.)
(Summary Experiment 5)

Lot No.	Week 1	Week 2	Week 3	Week 4	Week 5
A (Control)	42.7	63.3	80.9	63.9	96.8
A_1 (Control)	41.7	59.0	75.8	78.6	95.2
B (1 Mrad)	44.3	62.7	73.7	78.3	91.9
B_1 (1 Mrad)	37.1	57.1	68.8	82.1	90.3
C (2.25 Mrad)	45.6	65.5	81.3	93.0	98.2
C_1 (2.25 Mrad)	41.1	61.6	77.7	88.1	109.5
D (3.5 Mrad)	41.5	66.8	66.7	74.1	106.0
D_1 (3.5 Mrad)	39.7	55.0	63.6	75.3	95.7

* Average of 10 birds per lot unsexed

ANALYSIS OF VARIANCE

Appendix Table (xxxiii) - Average Rate of Gain
(Summary Experiment 5)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	3	400.21	133.40	6.75	3.13	5.01
Replicate	1	648.84	48.84	2.47	4.38	8.18
Weeks	4	13998.39	3499.60	177.20	2.90	4.50
Treatment x Weeks	12	507.58	42.30	2.14	2.31	3.30
Error	19	375.16	19.75			
Total	39	15330.18				

Duncan's Multiple Range Test

Treatment (Mrad.)	2.25	2.25	3.5	1.0	Control	Control	1.0	3.5
Rate of Gain (gms.)	76.72	75.60	71.02	70.18	70.06	69.52	67.08	65.86

Appendix Table (xxxiv) - Average Feed/Gain Ratio*
(Summary Experiment 5)

Lot No.	Week 1	Week 2	Week 3	Week 4	Week 5
A (Control)	1.06	2.15	2.24	3.55	2.81
A_1 (Control)	1.63	2.31	2.39	2.31	2.86
B (1 Mrad)	2.05	1.81	2.46	2.61	2.96
B_1 (1 Mrad)	1.83	2.38	1.98	2.21	3.27
C (2.25 Mrad)	1.74	1.73	2.23	2.68	2.77
C_1 (2.25 Mrad)	1.66	2.58	2.34	2.32	2.69
D (3.5 Mrad)	1.64	2.04	2.72	2.45	3.00
D_1 (3.5 Mrad)	1.14	2.06	2.85	3.01	2.13

* Average of 10 birds per lot unsexed

ANALYSIS OF VARIANCE

Appendix Table (xxxv) - Average Feed/Gain Ratio
(Summary Experiment 5)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	3	0.04	0.013	0.10	3.13	5.01
Replicate	1	0.02	0.02	0.16	4.38	8.18
Weeks	4	7.32	1.83	14.19	2.90	4.50
Treatment x Weeks	12	1.54	0.128	0.99	2.31	3.30
Error	19	2.45	0.129			
Total	39	11.37				

ANALYSIS OF VARIANCE

Appendix Table (xxxvi) - Muscle Thiamin Levels
(Summary Experiment 5)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	3	0.08	0.027	0.54	9.28	29.46
Replicate	1	1.13	1.13	22.60	10.13	34.12
Experimental Error	3	0.15	0.05			
Sampling Error	32	1.04	0.033			
Total	39	2.40				

ANALYSIS OF VARIANCE

Appendix Table (xxxvii) - Muscle Riboflavin Levels
(Summary Experiment 5)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	3	0.11	0.037	0.93	9.28	29.46
Replicate	1	0.07	0.07	1.75	10.13	34.12
Experimental Error	3	0.12	0.04			
Sampling Error	32	0.17	0.005			
Total	39	0.47				

ANALYSIS OF VARIANCE

Appendix Table (xxxviii) - Liver Thiamin Levels
(Summary Experiment 5)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	3	5.00	1.67	0.88	9.28	29.46
Replicate	1	15.14	15.14	7.97	10.13	34.12
Experimental Error	3	5.69	1.90			
Sampling Error	32	11.44	0.36			
Total	39	37.27				

ANALYSIS OF VARIANCE

Appendix Table (xxxix) - Liver Riboflavin Levels
(Summary Experiment 5)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	3	41.67	13.89	8.57	9.28	29.46
Replicate	1	9.59	9.59	5.92	10.13	34.12
Experimental Error	3	4.87	1.62			
Sampling Error	32	48.15	1.51			
Total	39	104.28				

ANALYSIS OF VARIANCE

Appendix Table (xl) - TBA Test - Mg. M.A. per gm. fat.
(Summary Experiment 6)

Source of Variation	DF	SS	MS	F	P.05	P.01
Treatment	4	0.132	0.033	2.20	3.84	7.01
Replicate	2	0.028	0.014	0.93	4.46	8.65
Experimental Error	8	0.116	0.015			
Sampling Error	60	0.213	0.004			
Total	74	0.489				