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**A STUDY OF THE NUTRITIVE VALUE
OF NEW ZEALAND MEATMEALS**

A Thesis Presented in Partial Fulfilment
of the Requirements for the Degree of
Master of Agricultural Science
in the Victoria University of Wellington.

by

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February 1963

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ACKNOWLEDGEMENTS

The author wishes to thank his supervisor Dr. E. L. Richards for his encouragement and advice on this study. The advice and guidance of Dr. D. S. Flux in statistical matters is also gratefully acknowledged. Special thanks is due to Mr. J. H. Kissling of the College poultry unit and his staff for their valuable help in this study.

Thanks are also due to: Mr. A. E. Esler and Mr. M. R. Grimmett for their assistance with the photography; to Miss B. Handley for her assistance with the chickens; to Miss M. G. Campbell and her library staff for their efforts in obtaining numerous journals.

Special thanks is due to Miss F. Fairweather for typing this thesis.

For the duration of this study the author was assisted by the Shell Oil Company and University Senior Scholarships.

INTRODUCTION

Meatmeals are an important source of protein in New Zealand poultry diets. These meatmeals are processed from the waste by-products of the freezing works and because of their high protein content they are a useful source of supplementary protein. It appears, however, that the meatmeals as a foodstuff are not uniform in their nutritive value, as poultry men have observed that young poultry grow well on some meatmeals and poorly on others. This observation has been confirmed by recent work (McDonald, 1961) which has been done in Australia.

The present study has been undertaken to investigate some of the factors influencing the nutritive value of the meatmeals and to find a chemical method which could be used to evaluate their protein quality.

CHAPTER I

REVIEW OF LITERATURE

A. The Meatmeals:

(1) The Composition of the Meatmeals.

The meatmeals are heterogenous products made by the processing of waste offal materials such as meat scraps, offal viscera, blood, skin and bone, etc. The variation of the protein content found in meatmeal can largely be explained in terms of the extent to which the fat has been removed from the product and the bone which has been added to the materials used in the manufacture of meatmeal.

The quality of the meatmeal protein or the nutritive value of the meatmeal is influenced by the composition of the raw materials used in the processing of the meatmeal. Considerable differences in digestibility and nutritive value of the protein of different organs and tissues has been clearly shown (Hoagland, et al. 1926A; Hoagland, et al. 1936). For example, the low nutritive value of tissues high in connective tissue such as hog snouts and calf lungs was demonstrated by the poor growth rate measured in rats when they were fed these materials in experimental diets.

Finally a comparison between the nutritive values of beef, calf lungs, hog spleens and cow udders showed that the nutritive value of beef was superior when compared to other organs which could be ranked in terms of their nutritive value according to the amount of connective tissue they contained (Hoagland, et al. 1926B). Therefore, the use of waste meat offals which are high in connective tissue for the manufacture of meatmeals will result in the production of meatmeals of inferior nutritive value. This is because the connective tissues are made up of the proteins collagen and elastin which are extremely deficient in several of the essential amino acids as shown in Table I (Sahyun, 1948).

TABLE I

The Approximate Amino Acid Content of Gelatin and Elastin (N X 6.25)

Amino Acid	Collagen Gelatin %	Elastin %
Arginine	8.8	0.9
Histidine	1.0	0.0
Lysine	4.5	-
Tyrosine	0.3	1.5
Tryptophan	0.1	0.0
Phenylalanine	2.1	3.1
Cystine	0.1	0.2
Methionine	1.0	0.4
Leucine	3.7	-
Isoleucine	1.7	-
Valine	2.1	-
Threonine	1.5	2.5
Glycine	23.6	27.5
Alanine	9.2	0.0
Serine	3.3	-
Glutamic acid	10.3	2.5
Aspartic acid	5.9	0.0
Hydroxyproline	13.0	1.9
Proline	15.3	14.2

(2) The Processing of Meatmeal.

In New Zealand the two processes used to process the meatmeal are:

- a. the wet rendering process.
- b. the dry rendering process.

In the wet rendering process the meat refuse is covered with water in large tanks and boiled until the tissue breaks down after which the fat is extracted. The material remaining is then dried and ground to give the final product known as meatmeal. In the dry rendering process the meat refuse is cooked without adding water in a steam jacket vessel which is heated by passing steam between the inner and outer walls of the jacket. After the fat is extracted from the broken down tissues the moisture is driven off and the material dried and ground. The actual temperatures used in the processing and the time the materials are cooked for varies between some freezing works.

A comparison between the two processes in terms of their effect on the nutritive value of the products has been examined (Pope, 1929), and the results of this study showed that there appeared to be no difference between the wet rendering and the dry rendering processes on the nutritive value of the protein of the products produced. In the case of fishmeal it was also shown that there was very little difference between the two processes. (Wilder, et al. 1934).

(3) The Amino Acid Content of Meat By-products.

The results of the amino acid determinations on meat by-products made by microbiological assays are shown in Table II (Lyman, et al. 1956; Almquist, 1957).

TABLE II
The Amino Acid Content of Meat-By-products.
(per cent of feedstuff)

Amino Acid	Meat Scrap			Meat Scrap		
	(1)	(2)	Tankage	(1)	(2)	(3)
Arginine	3.38	3.36	1.40	3.9	3.5	3.2
Histidine	0.81	0.74	2.05	0.97	0.88	0.80
Isoleucine	1.48	1.50	1.40	1.87	1.70	1.53
Leucine	2.96	3.04	5.74	3.5	3.2	2.8
Lysine	2.49	2.68	4.40	3.8	3.4	3.1
Methionine	0.64	0.66	0.76	0.71	0.65	0.58
Phenylalanine	1.73	1.64	0.78	2.04	1.85	1.67
Threonine	1.64	1.53	2.00	1.82	1.65	1.49
Tryptophan	0.33	0.32	0.61	0.36	0.35	0.27
Valine	2.38	2.17	4.18	2.75	2.50	2.25
Protein %	45.34	48.41	61.02	55	50	45
		Lyman			Almquist	

No figures on the amino acid content for New Zealand Meatmeals have been published.

The Amino Acid Composition of Meat.

Microbiological methods were used to estimate the amino acids present in meat (Hier, et al. 1945; Beach, et al. 1943, and Schweigert, et al. 1944).

These results are summarised in Table III.

TABLE III

The Amino Acid Content of Meat.
per cent of the protein (N x 6.25)

	Beef	Pork	Lamb	Veal
Arginine	6.4	6.4	6.2	
Histidine	3.9	3.8	3.2	3.5
Lysine	8.9	8.7	8.8	
Leucine	7.6	7.2	8.1	7.6
Isoleucine	5.7	5.7	6.4	5.6
Valine	5.3	5.5	5.4	5.3
Methionine	2.5	2.4	2.4	
Cystine	1.4			
Threonine	4.5	4.5	4.8	3.9
Tryptophan	1.4	1.4	1.4	1.1
Phenylalanine	4.2	4.2	4.3	3.8
Glutamic acid	14.2	11.8	11.9	
Tyrosine	3.4			
Glycine	5.0			

The analysis shows that meat is nutritionally and biologically complete since it contains all of the essential amino acids in adequate amounts.

However, amino acid supplementation experiments with beef (Mitchell, et al. 1932) have shown that cystine and methionine are the limiting amino acids in meat.

(4) A Comparison of the Amino Acid Content of the Meat Scrap Protein and Meat Protein.

TABLE IV
The Amino Acid Content of Meat and Meat Scrap.
per cent of protein (N x 6.25)

	Beef	Pork	Lamb	Meat Scrap (a)	Meat Scrap (b)
Arginine	6.4	6.4	6.2	7.46	6.94
Histidine	3.9	3.8	3.2	1.79	1.53
Isoleucine	5.7	5.7	5.4	3.26	3.10
Leucine	7.6	7.2	8.1	6.53	6.28
Lysine	8.9	8.7	8.8	5.49	5.54
Methionine	2.5	2.4	2.4	1.41	1.36
Phenylalanine	4.2	4.2	4.3	3.82	3.39
Threonine	4.5	4.5	4.8	3.62	3.16
Tryptophan	1.4	1.4	1.4	0.73	0.66
Valine	5.3	5.5	5.4	5.25	4.48

It appears that the meat scrap protein as shown by an examination of Table IV is lower in all of the essential amino acids, except arginine. This difference could be due to several factors.

1. The materials used in the manufacture of meatmeals are low in essential amino acids. Support for this idea was demonstrated by the fact that the nutritive value of the protein of the various offal materials used in the production of meatmeal was lower than the nutritive value of meat. (Hoagland, et al. 1926B). Also these offal materials are high in connective tissue which is deficient in essential amino acids.
2. The effect of the high temperatures used in the processing of these meatmeals may bring about a destruction or a reduced availability of some of the essential amino acids to the micro-organisms used in the determination of the amino acids.

B. The Effect of Heat on the Nutritive Value of Proteins of Animal Products.

(1) Fishmeals.

A study (Glandinin, 1949) on the effect of heat on the nutritive value of animal products was carried out using fishmeals which were dried under various degrees of vacuum for varying time intervals and by the flame method with stack temperatures of 185°F and 220°F. When the meals were fed as a source of supplementary protein in the diets of chickens it was found that there was a definite reduction in the nutritive value of the fishmeal dried at 220°F, as compared to the fishmeal flame dried at 185°F, and the vacuum dried fishmeal. These results were confirmed by the work of Elmslie, et al. (1957) which is summarized in the following table.

TABLE V.

Character of fishmeals.	Rat gain.	Chick weight at 6 weeks.
Normal meal	-	1.8 lb
Slightly overheat	74 gms/week	1.7 lb
Strongly overheat	36 gms/week	1.5 lb

(2) Meat.

The effect of heat on the nutritive value of pork (Beuk, et al. 1949) was demonstrated by the study of pork samples autoclaved at 113°C for 5, 15 and 30 minutes, and for 1, 2, 4, 8 and 24 hours. The effect of the heat treatment on the pork samples was measured in terms of weight gains of rats in whose diets the various treated samples were mixed. The rats fed on the raw pork gave the greatest gain in weight and the highest protein efficiency. A comparison between the raw meat and the meat autoclaved for five minutes showed a lower weight gain in the case of the autoclaved meat (significant difference at the 5% level), but the differences in the protein efficiencies were not significant. The comparison between the raw meat and the meat samples autoclaved at 2 and 24 hrs., however, showed a significant difference in weight gains and protein efficiency.

These results are confirmed by the work of Morgan, et al. (1934).

Finally, to summarize, it is clearly shown from the results of the above experiments that severe heating has a detrimental effect on the nutritive value of fishmeals and meat.

C. The Mechanisms Responsible for the Reduction in the Nutritive Value of Heated Proteins.

(1) The Role of Lysine in the Reduction of the Nutritive Value of Heated Proteins.

The effect of overheating on proteins has been subjected to considerable study and the mechanisms involved are slowly being elucidated. It has been shown that the nutritive value of heat damaged proteins can be improved by the supplementation with lysine in many cases. For example, the growth rate of rats fed on a diet containing heat treated casein was improved by the addition of lysine. (Greaves, et al. 1938). This has been confirmed by other work (Block, et al. 1946), which clearly demonstrated the deleterious effect that dry heat has on the availability of lysine in the dietary protein and the resulting decrease in the nutritive value of the foodstuffs. Supplementation experiments with lysine in the diets of chickens showed that the lysine was not destroyed when it was autoclaved for 4 hours at 120°C. However when the lysine was autoclaved under the same conditions with cerelese which is composed mainly of glucose, the growth rate of the chickens was found to be poorer due to the fact that the lysine had been destroyed, or rendered unavailable to the chicken. (Stevens, et al. 1947).

Two mechanisms are involved in the nutritional destruction of amino acids (Almquist, 1957).

- (i) The severe overheating of rich protein materials such as fish, meat and casein, which contain little or no free carbohydrate, causes the amino acid lysine and possibly arginine and histidine to become tied up by new chemical linkages to other amino acids, and so reduce their availability to the animal. This type of non-availability or retention of the amino acids will be termed the non-carbohydrate retention reaction.

- (ii) The overheating of proteins in the presence of carbohydrates initiates a series of reactions called the Maillard reaction or non-enzymic browning. The Maillard reaction involves the amino acids, particularly lysine, and to a lesser extent arginine and tryptophan, which react with the carbohydrates and undergo a series of reactions to give rise to the brown products of the Maillard reaction. These reactions result in some permanent chemical loss of the amino acid since they cannot be wholly regenerated by chemical hydrolysis. The net effect is a reduction in the availability of the amino acids to the animal, and a lowering of the nutritive value of the protein.

(2) The Non-Carbohydrate Retention Reaction.

(a) Discussion on the Non-Carbohydrate Retention Reaction.

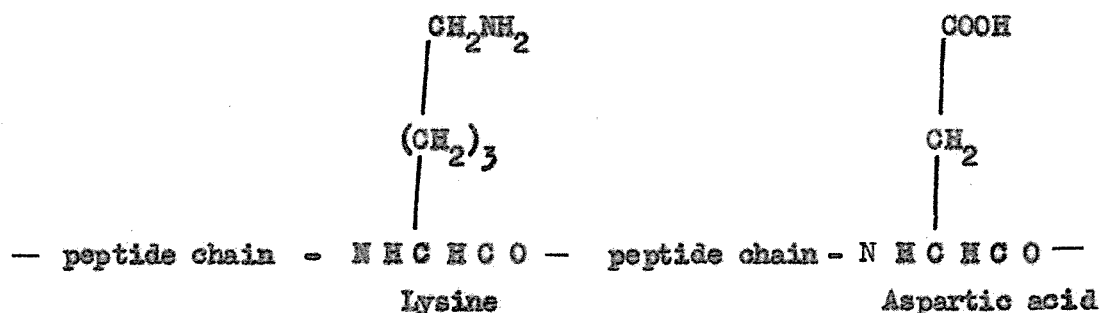
The reactions involved in non-carbohydrate retention do not bring about the destruction of the amino acids, because on acid hydrolysis the amino acids can be recovered as shown in the case of casein (Block, et al. 1934), and pork (Beuk, 1948). In the study with pork the samples of raw and cured pork were autoclaved at 112°C for 24 hrs. and the analyses of the acid or the alkali (in the case of tryptophan) hydrolysates of the autoclaved samples indicated a complete recovery of lysine, arginine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan and valine. A low apparent recovery was observed when the autoclaved samples were hydrolysed with enzymes. The treatment of these enzyme hydrolysates with acid permitted the recovery of the initial amount of all the amino acids, and this low apparent recovery in the case of the enzymes was probably due to the fact that the enzymes were not able to liberate all of the amino acids in the autoclaved pork samples.

The above findings were further confirmed by the observation, that microbiological assays on acid hydrolysates of heat damaged proteins showed no difference in the microbiological activity when compared with the acid hydrolysates from unheated protein (Pader, et al. 1948).

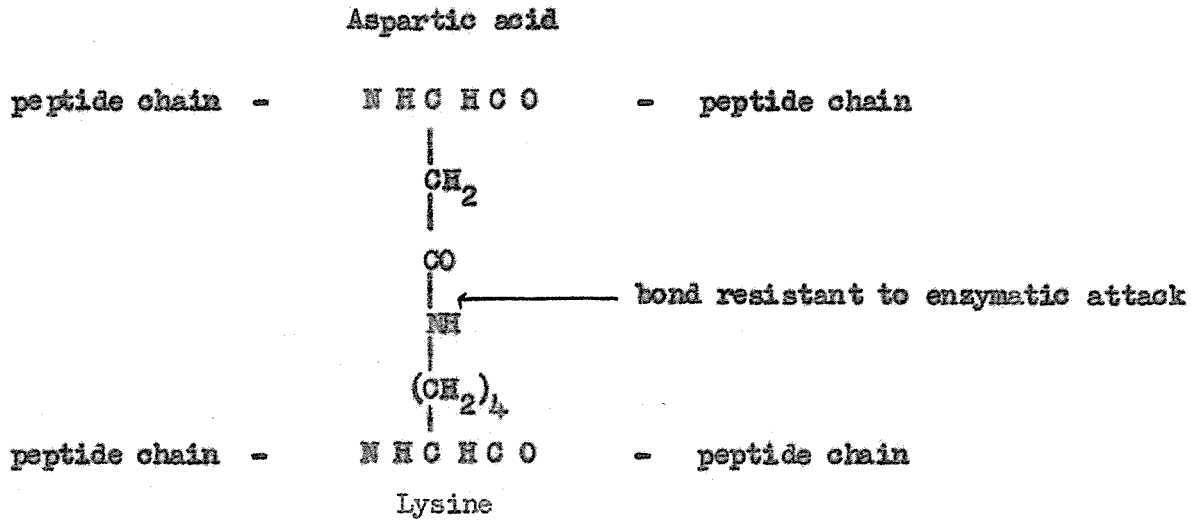
(b) The Postulated Mechanism Involved in the Non-Carbohydrate Retention Reaction.

It has been postulated (Pader, et al. 1948) that the effect of heat processing on the protein molecules brings about the formation of a complex linkage of the lysine with other active groups. These linkages are resistant to enzymatic attack, or the rate of the hydrolysis of the linkage was such that the lysine enters the blood stream too late to participate with the rest of the assimilated amino acids in the formation of protein, part of which would be used in building up new tissues. The linkage was thought to be a condensation between the free epsilon amino groups of lysine and the free carboxyl groups (Eldred, et al. 1946). These carboxyl groups could be contributed by the amino acids such as glutamic and aspartic acids, or the C terminal end of the peptide chains.

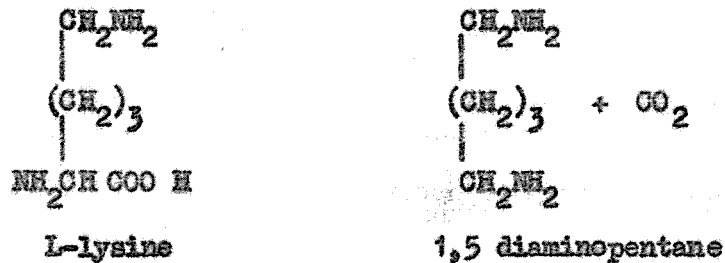
(i) before heating



(ii) after heating



Evidence for this theory comes from an experiment (Eldred, et al. 1946) which was designed to study the availability of lysine in raw and heated casein. In this experiment the enzyme L-lysine decarboxylase was used in the analysis of the L-lysine whose availability to this enzyme was found to decrease as the casein was more severely heated. L-lysine decarboxylase is specific in its action and will only decarboxylate lysine as shown in the following equation.



It will not attack α -acetyl —, α -methyl —, ϵ -acetyl —, ϵ -methyl lysine, or piperidine α -carboxylic acid. A change in the ϵ or α amino group of lysine would, therefore, affect its availability to this enzyme.

The α amino groups and the carboxyl

groups are presumably bound up in the peptide linkages, which remain unaffected by heat, as shown by the digestion with the crystalline enzymes. Therefore, the ϵ -amino group would appear to be the group involved. The mechanism of retention of the lysine seems to be the formation of a linkage between the ϵ -amino group of the lysine and some other active group which protects the lysine from the attack of the L-lysine carboxylase. The unavailability of other amino acids to digestive enzymes as demonstrated in other studies (Block, et al. 1934; Beuk, 1948), may possibly be explained by a similar mechanism. It is well known that the amino acids such as tryptophan, arginine and histidine contain reactive nitrogenous moieties not involved in peptide linkages, such as indole, guanidyl and imidazole groups which are capable of combining with other reactive groups.

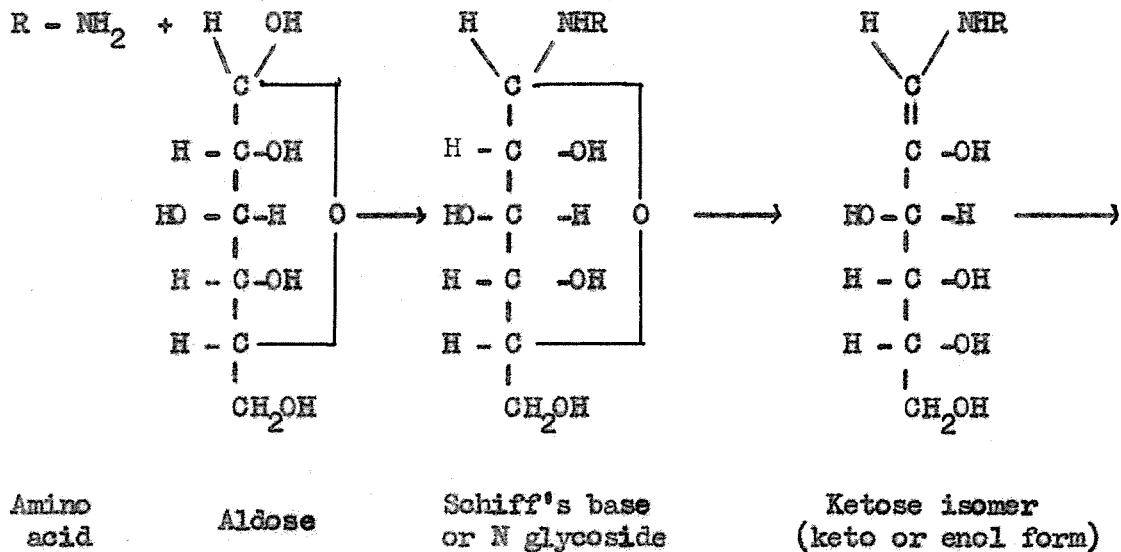
(3) The Maillard Reaction:

(a) The Reactions Involved and the Factors Influencing the Rate of the Reactions.

The Maillard reaction or non-enzymatic browning refers to the chemical reactions involving the amino acids and reducing sugars. The occurrence of the Maillard reaction is widespread especially during the processing of concentrated liquid foods and during their storage.

At the present time many of the intermediate products as well as the reactions involved in the Maillard reaction are not known, and the overall picture in this field is one of confusion. This has resulted from the multiplicity of conditions under which the reactions have been studied and the best attempt to rationalise the available information has been done by Hodge (1953) who distinguished three main stages.

The first stage involves a sugar amine condensation followed by an Amadori rearrangement. The sugars react with amino compounds to form a substituted glycosylamines or Schiff's bases (Gottschalk, et al. 1950), which are then converted to 1-amino-1-deoxy-2-ketoses. These may exist in the keto or the enol form (Richards, 1956). In general, it appears that -OH in the C₂ position of aldoses is essential for browning, as its blocking or absence precludes the Amadori rearrangement.



The second stage consists in sugar dehydration, sugar fragmentation, and either amino acid or amine degradation according to the compound combining with the sugar. Two main types of reaction occur in dehydration of the sugar moiety.

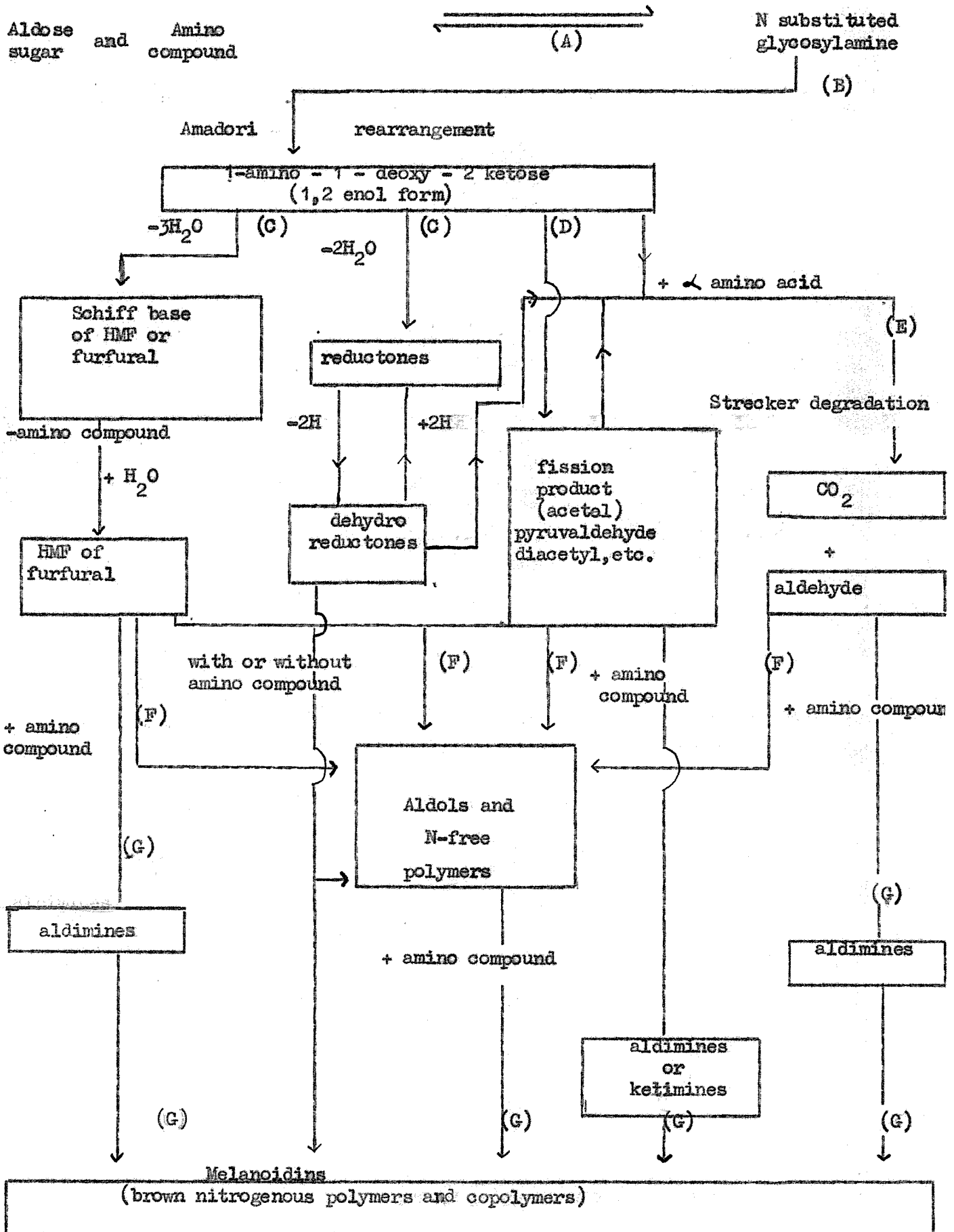
Also during the latter part of the second stage of browning fission products of the sugars appear, and the fragments which retain the α -carbonyl grouping will brown alone, but the discolouration was greatly increased by the presence of amino groupings in the reaction mixture (Carson, 1953). The Strecker degradation (Hodge, 1953) which involves the reduction of the α -amino acids to aldehydes (containing one carbon less than the amino acid with the liberation of CO_2) in the presence of reductones has some importance in the browning reaction, because the aldehydes formed are a source of browning. They are able to condense with themselves and with amines.

In the third stage, aldol condensations, aldehyde-amino polymerisation and formation of heterocyclic nitrogen compounds occur, but the pathways and reactions involved are not known. (Hodge, 1953).

A diagrammatic scheme of the reactions involved in the Maillard reaction is shown in Fig. I (Hodge, 1953).

FIGURE I

The Reactions of the Maillard Reaction.



- A. Sugar amine condensation.
- B. Amadori rearrangement.
- C. Dehydration of sugar moiety.
- D. Fragmentation of sugar moiety.
- E. Strecker degradation of amino acid moiety.
- F. Aldol condensation.
- G. Aldehyde-amine polymerization and formation of heterocyclic nitrogen compounds.

A study (Lea, 1958) of the mechanism in a "dry state" casein and glucose system showed that in the initial stages of the reaction there was an extensive combination of the sugar with the amino groups of lysine, and as the reaction proceeded further arginine, histidine, tyrosine and methionine all became involved.

The main factors affecting the Maillard reaction were:
(Lea, 1958; Jones, 1956).

1. Temperature.

As the temperature increased the rate of reaction between the protein amino groups and the free sugars was also found to increase.

2. pH.

The rate of reaction was shown to increase approximately linearly with increasing pH from 3 to 8. The amino acids do not react in the cationic form, only slightly in the Zwitterion form and fully in the anionic form.

3. Relative Humidity.

The critical moisture varies from system to system. In general, systems of carbonyl compounds combining with the amino function of high molecular weight are in equilibrium with higher relative humidity at the point of maximum browning than reaction mixtures with relatively simple compounds. For example, in casein glucose systems, the maxima are of the order of 70-75% relative humidity, where as a complex system of mixed extractives of low molecular weight, such as fish muscle, had a maxima of the order of 10-30%.

4. Heavy metals.

A number of heavy metals, such as copper, were found to act as catalysts in the reaction.

(b) The Implications of the Maillard Reaction on the Nutritive Value of Proteins.

A study (Baldwin, et al. 1951) of the effect of heat on a casein dextrose mixture, using the micro-organisms Leuconostic mesenteroides p60 and Streptococcus faecalis to determine the essential amino acids, clearly showed that some of the amino acids were destroyed. The extent of the destruction was as follows:-

Arginine	95%
Lysine	80%
Tryptophan and Methionine	40%
Valine and Histidine	30%
Isoleucine, Leucine, Threonine and Phenylalanine	15-20%

As a result the nutritive value of the casein was greatly impaired. When casein was mixed with a dextrose solution (McInroy, et al. 1949), and autoclaved for 2 hours, a dark brown rubbery product was formed which brought about a rapid loss in weight to rats when it was included in their diet, while the autoclaved casein in the absence of dextrose solution, included in a similar diet, resulted in good weight gains.

It has been postulated (Patton, 1948) that the amino acid residues of lysine, arginine, tryptophan and histidine are especially subject to destruction because of the reactive nitrogenous moieties uncombined in peptide linkages. These are the epsilon amino, guanidyl, indole and imidazole groups. Further, the studies of Mahamond, et al. (1949) have shown that the above groups are involved in protein sugar complexes.

(c) The Maillard Reaction in Fish Products.

It has been suggested (Miller, 1956) that the reduced nutritive value of heated fish products can be explained by the formation of amino acid sugar complexes resistant to enzymatic attack. However other studies have concluded that for foods, in general, the evidence suggests that the browning reaction is only related to the problem, and is not the actual cause of the reduction in the nutritive value of heated fishmeals. (National Research Council: Food and Nutrition Research Board, 1950).

In model casein-glucose systems it has been shown that the free sugar declines in quantities equivalent to the fall in the number of reactive amino-groups. The concentration of carbohydrates in fish products is, however, not high enough to account for the reduction in the nutritive value of some samples of heated fillets that have been investigated, although it has been observed that free ribose is present in the tissues of certain species of fish, and that the development of browning is paralleled by a fall in the level of this sugar. An interesting observation was made on the availability of amino acid in fishmeals (Bissett, 1954) when it was shown that the season, as well as the heat treatment during processing, affected the availability of the amino acids. A difference was noted between the February and November samples. The February samples showed a greater impairment in their nutritive value, and it was also found that these meals contained a greater amount of deoxypentose nucleic acid and pentose. It was suggested that the Maillard reactions between liberated pentoses and amino acids may have been responsible for the observed differences.

D. The Effect of Non-Carbohydrate Retention and the Maillard Reaction on the Enzymatic Liberation of Lysine:

(1) The Maillard Reaction.

In a study (Lowy, et al. 1950) to demonstrate the influence of the Maillard reaction on the action of enzymes, autoclaved casein dextrose products were used as substrates for the following enzymes:- Trypsin, chymotrypsin, pepsin, papain and pancreatin. The results showed that the reaction products were resistant to digestion by trypsin and papain, while they were digested by pepsin, chymotrypsin and pancreatin. These observations lead

to the postulation of an idea that the loss of nutritive value was due to the protein-sugar complex being resistant to trypsin. However it must be pointed out that it is difficult to relate these in vitro studies to the animal. It is interesting to note that the site of attack for trypsin, which is a highly specific enzyme, is the peptide linkage on the carboxyl side of the amino acids, arginine and lysine and it is, therefore, probable that the reaction of lysine and arginine with the free sugar through their ϵ -amino and guanidyl groups protect the peptide linkage from the attack of trypsin.

(2) Non-Carbohydrate Retention Reaction.

In vitro studies (Pader, et al 1948), with pancreatin, showed that the rate of hydrolysis on casein by this enzyme was reduced by heating the casein at 150°C for 1½ and 5 hours, while the ultimate degree of hydrolysis which was obtained was not affected by the heat treatment. Also it was found that the supplementation of the heated casein with lysine restored the impaired nutritive value. These observations clearly indicated that the heating of casein affected the rate of liberation of the lysine, and it was postulated that the lysine was liberated too slowly to supplement effectively the amino acids which were more readily hydrolysed, and hence absorbed earlier during the period of digestion.

(3) The Availability of Lysine in Heat Processed Animal Products.

In a study on commercial samples of blood meals chickens were used to estimate the availability of lysine (Kratzer, et al. 1957). The basal ration used contained 20% protein which was supplied by sesame seed meal and corn, and the blood meals were added to the experimental diets at the expense of sesame seed meal and corn starch so as to keep the total protein level constant. Simple body weight gains for a 10-12 day period were taken as the measure of response and the results compared with lysine

values obtained by the microbiological assays of the acid hydrolysates of the blood meals. The comparison showed that for the three samples prepared by careful spray drying at 145°F the mean availability of the lysine for chicks was 75%, and in the case of the two vat dried meals the mean availability was 65%.

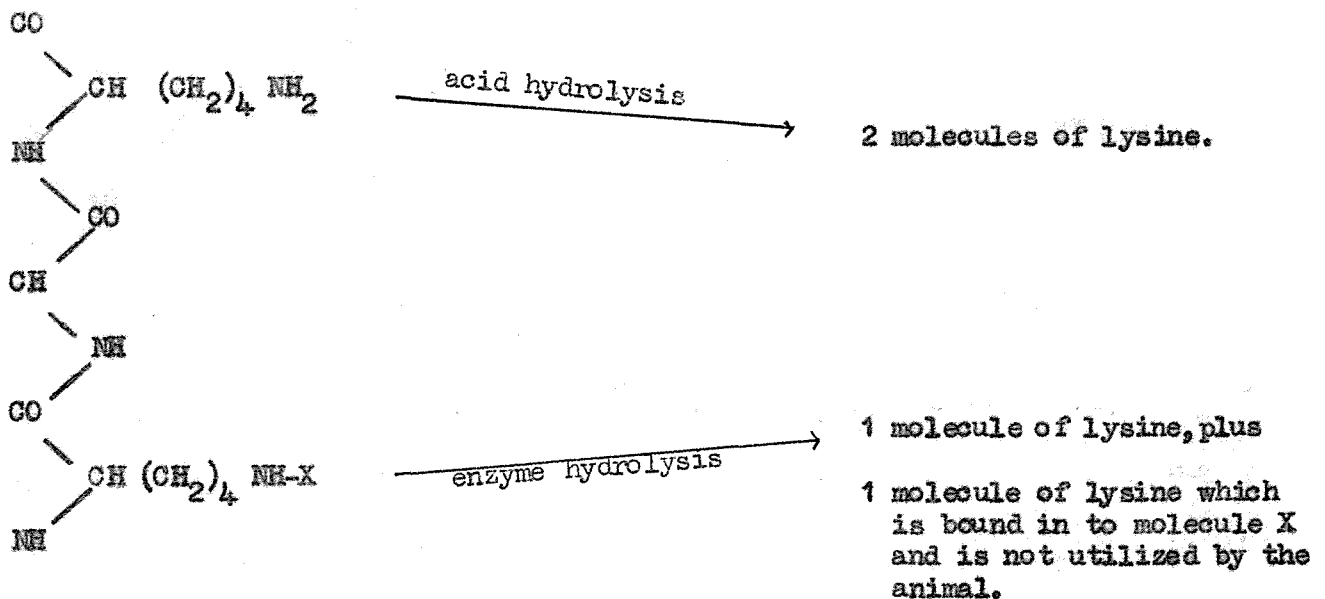
The method of processing herring meals also influences the availability of their essential amino acids (Clandinin, 1959), and a comparison of the microbiological assays on the acid and alkali hydrolysates (in the case of tryptophan) with the enzymatic hydrolysis measured the extent to which the amino acids were retained. The results showed that the liberation of all the essential amino acids by enzymatic hydrolysis was greatly depressed, as the temperatures used in processing increased from 185°F to 200°F. Of the individual amino acids, lysine was the most seriously damaged, while the amino acids, arginine and tryptophan, were the least affected.

E. The "Available Lysine" and its Estimation:

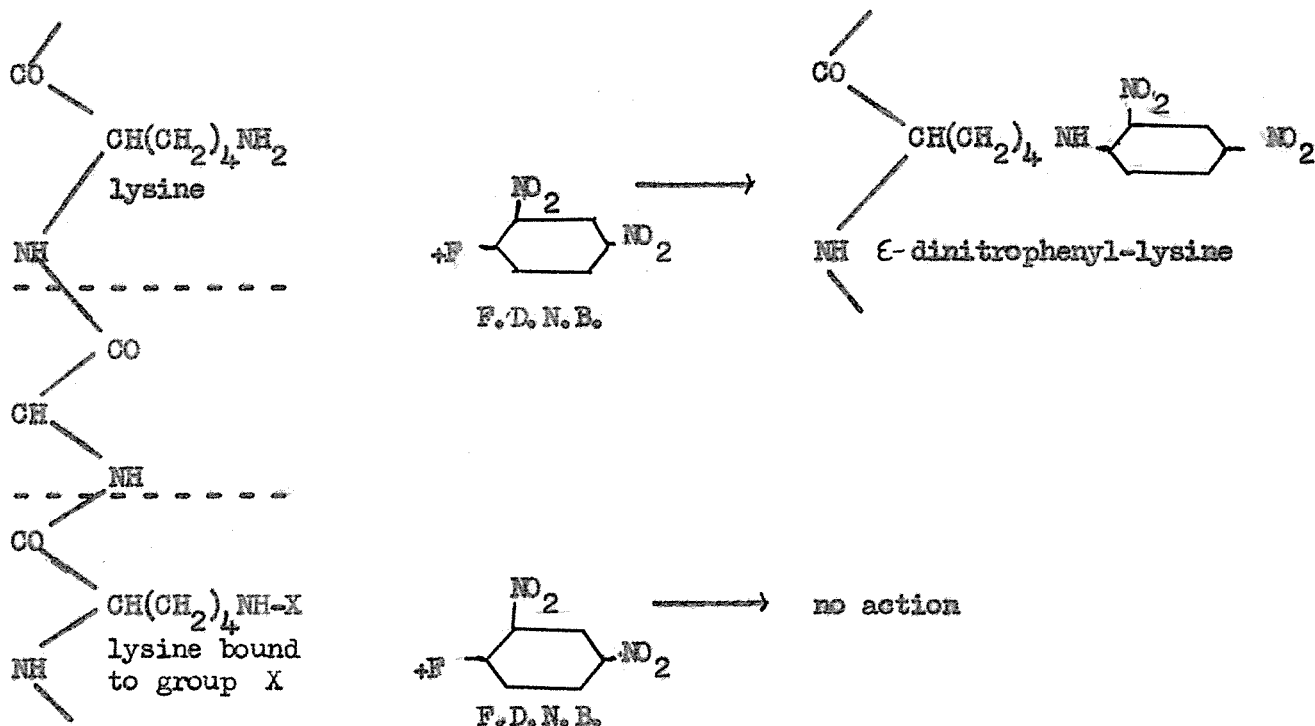
The above experiments show that lysine is definitely very vulnerable to "damage" in foodstuffs which require heat during processing. The loss of lysine can be explained by the fact that in heat damaged protein the ϵ -amino group of the lysine forms a linkage with other active groups which is resistant to the action of enzymes. The lysine whose ϵ -amino groups are not involved in complex linkages is termed the "available lysine". If the lysine was damaged to such an extent that it became the limiting amino acid, or its damage paralleled the destruction of the limiting acid, then the estimation of the "available lysine" would give a measure of the nutritive value of the protein in heat treated foodstuffs.

A method (Carpenter, 1960) has been developed to determine the "available lysine" in animal products, and the procedure involves the use of 2, 4-dinitrofluorobenzene (F.D.N.B.) which reacts with the free ϵ -amino groups of the intact protein. The "available lysine" in the intact protein has free ϵ -amino groups which are not involved in peptide linkages, and are able to react with the F.D.N.B. On acid hydrolysis of the protein the ϵ -dinitrophenyl lysine is released and its concentration can then be determined after purification, colorimetrically. Thus the F.D.N.B. technique gives a measure of the lysine which has not reacted with other active groups and is available to the animal for metabolism. A schematic diagram to illustrate the difference between results by acid hydrolysis and by biological assay for the lysine content of processed foods is shown in Fig. 2. (Carpenter, et al. 1957).

Figure 2: The Difference between Results by Acid Hydrolysis and Enzymes.



The F.D.N.B., like the enzymes, does not react with the NH_2 groups bound to groups like X, and the net result is that only one molecule of lysine reacts with the F.D.N.B.



Using the F.D.N.B. technique for the estimation of "available lysine" (A.V.L.) in animal products, such as fish and whale products, a relationship between the "available lysine" and the gross protein value (G.P.V.) was found. (Carpenter, et al. 1955). The G.P.V. is a measure of the nutritive value of the animal products calculated from the growth rate of chickens. (Carpenter, et al. 1955). The statistical analysis showed the relationship between the G.P.V. (Y) and the A.V.L. (X) to be highly significant, and the following regression equation was worked out.

$$Y = 43.8 x - 2.69 x^2 - 75.5$$

The correlation calculated between the G.P.V. and A.V.L. was found to be $r = 0.97$.

F. Supplementation of Meatmeals with Amino Acids:

To investigate the limiting amino acids in the meatmeals, experiments involving the supplementation of these meatmeals with amino acids such as lysine, methionine and tryptophan were carried out (March, et al. 1950; Patrick, 1953). In these experiments the meatmeals were supplemented with methionine, tryptophan and lysine individually, and in various combinations, after which they were fed with a basal cereal ration to chickens. The results showed that lysine was the limiting amino acid, while methionine was the second limiting amino acid under the conditions of the experiments.

The above experiments were criticized on the grounds that the basal diets used were deficient in lysine, and the protein of the basal diets contributed to the protein fraction under investigation. In a second series of experiments (Kratzer, et al. 1959), in which the ten samples of meatmeal examined formed the major source of the protein in the experimental diets, except for 2% fish solubles which supplied the other source of protein, different results were obtained. The results of this work indicated that the meat meals were deficient in methionine and tryptophan and not lysine, and these observations were in agreement with the work of Wilder, et al. (1947).

G. The Influence of Varying Calcium and Phosphorus Levels on the Growth Rate of Chickens:

The ash content of the meatmeals varies greatly and the importance of this component as a probable factor influencing the nutritive value of the meatmeals is shown by the fact that the ash contains a high percentage of calcium and phosphorus. Therefore the use of meatmeals in formulating rations would give rise to diets of varying calcium and

phosphorus contents. Recent work has been done (Simco, et al. 1961) to examine the effect of varying the levels of calcium and phosphorus on the growth rate of chickens. The levels of calcium and phosphorus studied were 0.15%, 0.5%, 0.6%, 0.8%, 1% and 0.42%, 0.5%, 0.6% and 0.8% respectively. The results of this study showed that:

1. The calcium level of 0.15% did not support optimum growth or feed utilization.
2. There was found to be no significant difference in growth and feed utilization between diets containing 0.5%, 0.6%, 0.8% and 1% calcium in presence of adequate phosphorus.
3. The phosphorus level of 0.42% did not support optimum growth or feed utilization.
4. There were no significant differences between the growth rate and feed utilization when phosphorus was fed at levels of 0.5% and 0.6%.
5. When the phosphorus was fed at a level of 0.42% the feeding of excessive calcium (1%) appeared to magnify the growth depression.
6. The optimum growth and efficiency of feed utilization was obtained with a calcium level as low as 0.5% and a phosphorus level of 0.5%.

Further, recent work (Formica, et al. 1962) found that the optimum growth rate was measured at 0.6% calcium and 0.6% phosphorus. Finally, some English work (Smith, et al. 1961) comparing two diets containing 0.83% and 1.35% calcium showed that the 0.83% calcium diet

promoted a faster growth rate and a higher feed conversion efficiency, and the differences were significant at 1% level.

Another important factor related to calcium and phosphorus is the ratio of these two elements in the diet. It appears that the ratio of 1.5 : 1 is the most desirable ratio (Morrison, 1959), but rations containing a calcium phosphorus ratio ranging from 1:1 to 2.5 :1 did not have any detrimental effects, whereas a 3.3 :1 ratio was found to be detrimental.

The percentage of fat in the diet may also influence the calcium requirements (Pepper, et al. 1955), as these requirements for maximum efficiency were found to increase when the diets contained 5% and 10% of fat.

H. An Outline of the Present Study on the Nutritive Value of N. Z. Meatmeals:

The following experiments were designed to obtain information on various facets of this problem.

Experiment I: A preliminary study of the factors affecting the nutritive value of N. Z. Meatmeals.

This experiment was first set up to see whether the nutritive value of the meatmeals was influenced by one or more factors as meatmeals are heterogenous materials, and the mineral content (calcium and phosphorus), as well as the protein quality, may influence the nutritive value.

Experiment II: To study the influence of the varying levels of calcium and phosphorus on the growth rate of the chicken.

Meatmeals contain varying amounts of calcium and phosphorus, and a factorial experiment was designed to study the effect of

varying the levels of calcium and phosphorus on the growth rate of the chicken under the conditions in which Experiment I was conducted.

Experiment III: A study of the effect of heat on the nutritive value and "available lysine" content of animal protein:

The heating of animal protein causes a loss in the nutritive value and a reduction in the "available lysine", and this experiment was designed to measure the extent of this damage under various conditions.

Experiment IV: To study the effect of supplementing meatmeal with L-lysine.

To demonstrate whether the reduction of "available lysine" during the processing of the meatmeal resulted in the lysine becoming the limiting amino acid, a sample of meatmeal was supplemented with lysine.

Experiment V: To study protein quality and the "available lysine" in N.Z. Meatmeals.

To study the possibility of a correlation existing between the "available lysine" content and the biological value of the meatmeal protein, as determined by the gross protein value method, the gross protein of each meatmeal was determined.

CHAPTER II

MATERIALS, METHODS & EXPERIMENTAL.

A. Materials:

(1) Meatmeals.

The eleven samples of meatmeal used in this study were obtained from a number of freezing works in the North Island and South Island. These meatmeals were stored in a deep freeze until required for the experiments.

The meatmeals were manufactured from the various by-products of the freezing works and were processed by one of two methods.

- a. the dry-rendering process.
- b. the wet-rendering process.

The actual conditions under which these meatmeals were processed are outlined in Appendix I.

(2) Autoclaved Meat.

One hundred and twenty-five pounds of minced lean beef was obtained and eighty pounds of this meat was treated in an autoclave, as follows:

- a. 5 minutes at 240°F.
- b. 25 minutes at 240°F.
- c. 2 hrs. at 240°F.
- d. 2 hrs. at 270°F.

The remaining forty-five pounds was not autoclaved. All samples of meat were freeze dried and put into cold storage.

B. Methods:

(1) The Autoclaving of Meat Samples.

The samples which were autoclaved were thinly spread out on trays before they were separated by blocks and placed in the autoclave bucket. This arrangement enabled the heat of the steam to penetrate the meat and so ensure that the samples were uniformly cooked. The water in the autoclave was allowed to boil before the samples were placed in the autoclave and brought up to the nominated temperature and held for the required autoclaving interval. When the autoclaving was finished the pressure was released as quickly as possible, after which the samples were removed and allowed to cool before they were stored in the deep freeze. The 45 lbs. of raw meat and the autoclaved samples were then freeze dried, but before returning to the deep freeze they were allowed to equilibrate with the moisture in the air.

(2) Sampling Procedure.

To sample the various constituents of the experimental diets, for the purpose of a chemical analysis, the bulk sample was spread out on the floor and quartered repeatedly until a sample of suitable size was obtained for the chemical analysis.

(3) The Methods Involved in the Chemical Analysis of the Constituents of the Experimental Diets.

All samples were analysed in duplicate. The freeze dried meat and meatmeal samples were analysed for moisture, ash, fat, protein, calcium, phosphorus and "available lysine". The wheat, millers' offal, maize, barley, oats, whey, yeast and casein were only analysed for protein, calcium and phosphorus.

a. Moisture.

2 gm samples were weighed out in aluminium dishes of known weight which had been previously warmed and cooled in a desiccator. The dishes with their lids removed were then placed in an oven for 16 hours at a temperature of 100°C. The lids were then replaced on the dishes which were then cooled in a desiccator before they were re-weighed. The loss in weight gave the hygroscopic moisture content.

b. Crude Protein.

The total nitrogen was determined by the Kjeldahl method. (Kjeldahl, 1833). The 0.4 gm samples used were digested in 100 ml Kjeldahl flasks with 20 mls of concentrated sulphuric acid, 10 gms of sodium sulphate and 10 mls of mercuric chloride. (Hiller, et al. 1948). The nitrogen was converted to the salts of ammonia during digestion and when this solution was boiled with 18N sodium hydroxide in presence of zinc, the ammonia which was released was collected in boric acid. (Meeker, et al. 1933). The ammonia released and, hence, the total nitrogen in the foodstuff was determined by a back titration with $\frac{N}{10} H_2 SO_4$. (1 ml $\frac{N}{10} H_2 SO_4 \equiv 0.0014$ gm nitrogen.) The crude protein was determined by multiplying the nitrogen content by 6.25.

c. Fat.

The contents of the aluminium dish used in the determination of the moisture content of the foodstuff were carefully transferred to an extraction thimble which was then placed in a soxhlet extractor. The soxhlet extractor was then fitted on to a flask containing 150 mls of anhydrous ether which was heated in a water bath held constant at 65°C. A condenser was fitted on top of the extractor and the extraction

was carried on over a period of 16 hours. After the extraction was completed all the ether was returned to the flask from the extractor, after which it was removed by distillation from the flask. To remove the last traces of moisture the flask was placed in an oven at 100°C for 1-2 hrs., and after cooling it was weighed. The flask was then re-weighed after the fat material had been removed, and the difference between the two weighings represented the fat material present in the sample.

d. Ash.

A 2 gm sample was weighed in a silica crucible of known weight, after igniting it and cooling it in a desiccator. The sample was then ashed in a furnace at 600°C for 2 hours. The temperature was controlled to prevent volatilisation of elements such as sodium and potassium. After ashing the crucibles are cooled in a desiccator and weighed to obtain the weight of ash.

e. The Determination of Calcium.

The ash from the crucible was transferred carefully into a 400 ml beaker. The crucible was then washed with 10 mls of 1:1 hydrochloric acid and 20 mls of water to remove any remaining ash particles into the beaker which was warmed to dissolve the ash. The calcium determination was carried out by the urea hydrolysis method. (Vogel, 1951). The calcium oxalate precipitate formed was dissolved in concentrate sulphuric acid, and the solution warmed to 70-80°C and titrated with $\frac{N}{10}$ K_{MNO_4} prepared, according to Vogel (1951).

$$1 \text{ ml } \frac{N}{10} \text{ } K_{MNO_4} \equiv 0.00200 \text{ gm Ca}$$

In the case of the meatmeals, which are high in calcium, only one-fifth of the solution obtained by dissolving the ash in hydrochloric acid was used in the calcium determination.

f. The Determination of Phosphorus.

A colorimetric method was used to determine the phosphorus content of the foodstuffs. The size of the sample which was digested for this micro-analytical method was found to be too small for the meatmeals, as it introduced sampling problems due to the presence of bone particles. To overcome this sampling problem a 2-3 gm sample was digested in a 250 ml Kjeldahl flask with 40 mls of analar concentrate sulphuric acid, plus a drop of anti-foaming agent. The Kjeldahl flask was heated gently at first to prevent excessive frothing. After the frothing had subsided the Kjeldahl flask was heated strongly for 3 hrs. The black solution was then decolourized by cautiously dropping into the boiling mixture from a dropping bottle analar concentrated nitric acid, until the solution turned a light yellow. The solution was allowed to boil for a further 15 minutes and after it had cooled the neck of the flask was washed with hot water, after which its contents were boiled for a further 15 minutes. On cooling the contents of the Kjeldahl flask were carefully transferred to a 1 litre volumetric flask, together with about 800 ml of water. When the volumetric flask contents had cooled, after the reaction between the water and acid, the solution was made up to the mark with water and mixed. From the 1000 ml volumetric flask, 1 ml of the solution was transferred to a 25 ml volumetric flask for the phosphorus determinations by the method of Allen (1940).

g. The Determination of "Available Lysine".

The "available lysine", or the lysine with the free ϵ -amino groups, was determined by the method of Carpater (1960), modified by replacing the third stage (after the extraction with ether to remove the ether soluble D.N.P. amino acids) by a chromatographic method for the separation of ϵ -D.N.P. lysine (Baliga, et al. 1959). The eluate

from the strip of paper chromatogram containing the ϵ -D.N.P. lysine was made up to 25 mls in a volumetric flask with hydrochloric acid, and its extinction coefficient was read at 435 m μ in a Beckman model D.U. Spectrophotometer. The concentration of ϵ -D.N.P. lysine was then read from a standard curve prepared using ϵ -D.N.P. lysine hydrochloride prepared by the procedure of Porter and Sanger (1948).

The recoveries of ϵ -D.N.P. lysine, when the meatmeals were analysed, were checked by the method of Carpenter (1960).

(4) The Experimental Brooder Layout.

(a) The Brooders.

In this study two brooder units were used. The brooder could be described as a large wheel with a diameter of 6 ft. surrounded by an 18 inch high wall, and containing a centrally placed circular heating unit. The brooder was divided into nine compartments by wooden partitions radiating from the centre of the brooder to the outside (Fig.3). Sawdust was placed on the floor of each compartment to a depth of $2\frac{1}{2}$ -3 inches, and each compartment contained a watering and feeding unit. Fitted to each brooder unit was a 35 watt light bulb to ensure equal illumination in all compartments, especially in the winter months. The two brooder units were housed inside a room in which the temperature and ventilation could be controlled.

(b) The Watering Units.

For the construction of the watering units, see Fig. 4.

(c) The Feeding Units.

The feeding unit (Fig.5) was constructed so that the feeding could be adjusted as the chicken grew, so as to control and prevent the spillage of the diet.

Fig. 3:

A General View of the Layout of the
Experimental Brooder.



(a) The experimental brooder



(b) The compartment.

Fig. 4:

THE WATERING UNIT



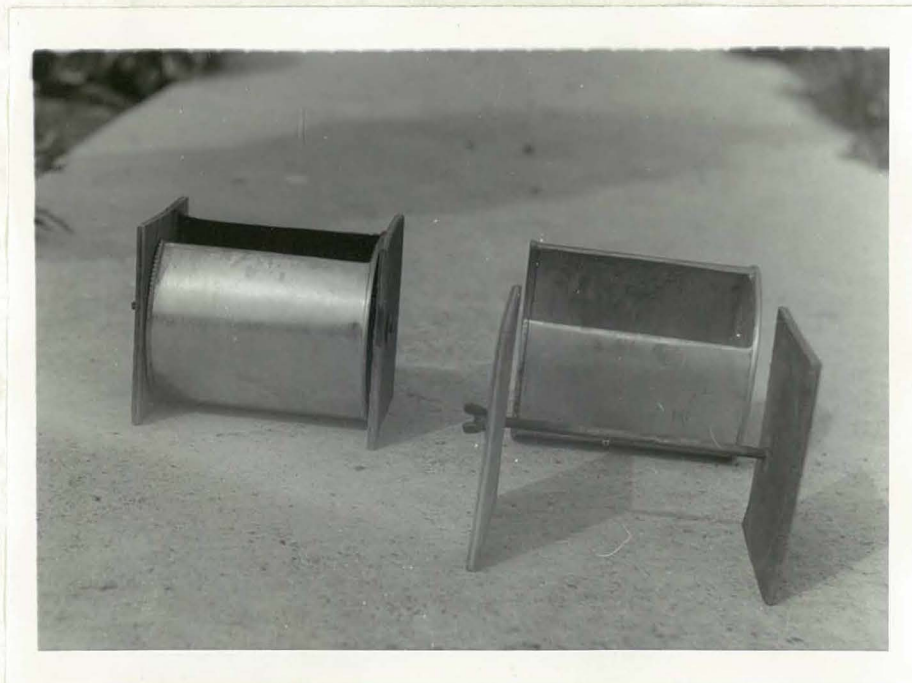
(a)

(b)

- (a) The assembled unit.
- (b) The components.

Fig. 5:

THE FEEDING UNIT



(a)

(b)

- (a) The assembled unit.
- (b) The components.

(5) The Management of the Chickens.

Chickens of the same line were used for all the experiments conducted in this study. For each experiment 250 day old chickens were wing banded and randomly placed in the eighteen compartments of the two experimental brooder units. During the whole experimental period the room temperature was maintained at 70°F., while the temperature under the brooder for the first two weeks was maintained at 90°F, after which the brooder heating units were raised to give a temperature under the brooder of 80°F. A continuous supply of clean drinking water was available at all times. Each experiment ran for 28 days and was divided up into a 14-day pre-experimental period and a 14-day experimental period.

(a) The Pre-Experimental Period.

To start the young chickens eating and to ensure that they were well supplied with grit, rolled oats were sprinkled on a thin layer of sand placed on a sheet of paper in each compartment. Since the first instinct of the chicken was to walk around and peck at anything that caught its eye, the spreading of the sand and the rolled oats on the ground ensured that the chickens would soon commence feeding, and at the same time receive adequate grit. At the end of the second day the feeding units containing the pre-experimental ration were placed in their respective compartments. These units were filled each morning so that the chickens were fed ad lib.

At 10 days of age all the chickens were debeaked. This involved the removal of part of the upper mandibular and the point of the lower mandibular. Debeaking was done as a necessary precaution against cannibalism which can start when the chickens are about 2-3 weeks old. The actual causes of cannibalism are not known, but a low protein diet could be a contributing factor.

At the same time as the debeaking all the chickens were identified by their wing bands and weighed. From this information the heaviest and the lightest birds were culled, and the rest of the chickens were then randomly divided into eighteen groups of ten chickens and placed in the compartments the following day. Heavy culling was done in order to obtain 18 groups of uniform body weight. This is important as the initial body weight influences the growth rate. The chickens were then allowed 3 days to settle down before the experiment was carried out. The loss of chickens during this pre-experimental period did not exceed 3%.

(b) The Experimental Period.

At 14 days of age the chickens were weighed and the basal diets replaced by the experimental diets which were randomly placed over the experimental groups of chickens. During the experimental period of 14 days the daily feed intakes were measured for each group, and at weekly intervals the individual chickens were weighed. During the experimental period a net was placed over each of the brooder units to keep the chickens confined in their compartments.

The management of the chickens outlined above was the same for all experiments in this study. At the finish of each experiment the sawdust was removed, the experimental brooder units dismantled and scrubbed, as were the water and feeding units of each compartment. Finally the room was washed out and the floor scrubbed with calcium hypochlorite.

This was done as a routine precaution against the possibility of the build up of disease, as the room was continuously used for these experiments for a period of 8 months.

(c) The Weighing Procedure used for the Chickens.

The chickens were weighed in a small wire basket placed on the pan of a Mettler K₇ balance with a scale graduated in units of 0.1 gm. The basket was tared to zero so as to give a direct reading of the chicken's weight. The weight of excreta excreted by the chicken is very small compared to the body weight of the chicken, and hence no problems arose in obtaining good estimates of the chicken's body weight if the chicken excreted before or during weighing.

(d) The Measurement of the Daily Feed Intake.

The chickens were fed each morning and the daily intakes were measured by the difference between the weight of diet put out in the previous morning, and the amount left in the feeding unit next morning which was weighed before the feeding unit was filled with a known weight of diet.

C. Experimental:

(1) The Experiments.

Experiment I: A Preliminary Study of the Factors Affecting the Nutritive Value of N.Z. Meatmeals.

This study was based on a bio-assay technique which enables the response in growth rate to the changing levels of supplementary protein to be measured.

Each netmeal was fed as a source of supplementary protein in the experimental diets which were fed at the three protein levels of 11%, 15% and 20%, and in each experimental diet the protein of the basal ration contributed 9.31% of the protein. The growth rate was plotted against the log of the

protein intake and the regression lines (growth rate on protein intake) were drawn. When all the regression lines for each meatmeal were plotted they were then analysed statistically for parallelism. If two or more regression lines were found to be parallel, then this showed that the meatmeals concerned belonged to the same population.

Each meatmeal tested was fed to 30 chickens divided up into 3 groups of 10 which were then allotted one group to each protein level. To test all the meatmeals two separate trials were conducted, and in order to check on any factors which may have influenced the results between the trials a standard meatmeal was run with each trial.

Experiment II: To Study the Influence of the Varying Levels of Calcium and Phosphorus on the Growth Rate of the Chicken.

Two trials were set up for this study. The first trial was a factorial experiment in which the effect of two levels of phosphorus (0.3% and 0.8%) and three levels of calcium (0.5%, 0.9% and 1.4%), at a protein level of 20%, on the growth rate of the chicken were studied. The second trial was designed to study the influence of increasing the level of calcium from 1.4% to 1.9% in a diet containing 0.8% phosphorus on the growth rate of the chicken. Each treatment in both trials was duplicated with 10 chickens per treatment. The data in the first trial was statistically analysed for significant differences between the levels of calcium and phosphorus and for the interactions between the levels of calcium and phosphorus. In the second trial the data analysed for significant differences was between the levels of calcium.

Experiment III: A Study of the Effect of Heat on the Nutritive Value and the "Available Lysine" of Meat.

The meat used in this experiment was subjected to the five treatments outlined below:

- (a) Freeze dried raw meat.
- (b) Autoclaved for 4 minutes at 240°F.
- (c) Autoclaved for 25 minutes at 240°F.
- (d) Autoclaved for 2 hrs. at 240°F.
- (e) Autoclaved for 2 hrs. at 270°F.

Treatment (e) corresponds to conditions similar to those experienced by the meatmeals during their processing.

The design of the experiment was the same as that of Experiment I. All treatments were fed as a source of supplementary protein in experimental diets which were fed at the protein levels of 11%, 15% and 20%, with the basal diet contributing to 9.31% of the protein. Each treatment was tested with 30 chickens divided up as in Experiment I.

The regression line of each treatment was plotted and the freeze dried meat was compared with each of the autoclaved meats. In each comparison the regression lines were analysed statistically for parallelism and for significant differences in elevation.

Experiment IV: To Study the Effect of Supplementary Meatmeal with L-lysine.

To test only the meatmeal protein and to reduce the influence of the mutual supplementary action of the other proteins which would influence the result obtained, the basal diet used in Experiments I, II and III was replaced

by a synthetic basal diet containing only 3.88% protein.

The lysine supplemented and the lysine unsupplemented meatmeal was fed at two levels of protein (15% and 20%) with the basal diet only contributing 3.88% of the protein. The lysine supplemented meatmeal and the lysine unsupplemented meatmeal were each tested with 20 chickens which were divided into 2 groups of 10 - one on each level of protein.

The regression lines were plotted and statistically analysed for parallelism and a significant difference between them which would measure the supplementary effect of the lysine.

Experiment V: A Study of the Protein Quality and the "Available Lysine" in N. Z. Meatmeals.

The gross protein value (G.P.V.) was determined by a modification of the method used by Carpenter, et al. (1955). The modification was made in the composition of the cereal components of the basal diet which contained adequate levels of minerals and vitamins and contributed 8% of the protein in the experimental diet. The test protein was fed at a level of 3% to make the final protein level in the experimental diet to 11%. The seven samples of meatmeal, the casein which was used as the standard, and the basal diet, were each fed to 2 groups of 12 chickens. The 18 experimental groups were randomised in the two experimental brooders.

The "available lysine" content of each meatmeal was determined by the method of Carpenter, (1960) with the modifications of Baliga, et al. (1959). The "available lysine" and gross protein value data were then used to compute a correlation and a regression equation.

(2) The Experimental Diets.

(a) The Preparation of the Diets.

The wheat, maize, barley and oats were finely ground so they would mix in with the other components of the diet and form a suitable dry mash for the experimental diets. Also because ground cereals lose their palatability on ageing these were only ground as required. The commercial dried yeast used in Experiment IV was first heated for 1 hour at 100°C and then ground before it was mixed in the experimental diet. This was done to kill the yeast cells which otherwise may begin to ferment in the digestive tract and cause digestive upsets.

A standard procedure was adopted in the mixing of all diets. The various components of the diet were weighed out separately and the mixing done in stainless steel trays which held 10 kilograms of diet. Portions of the major components of the diet were spread out evenly on the tray followed by portions of the other components of the diet, except the cod liver oil. This layer procedure was repeated using all the components of the diet after which they were thoroughly mixed by hand. The cod liver oil was then mixed into the diet. This involved mixing the cod liver oil required with a small part of the diet and then gradually mixing in the rest of the diet.

The various diets were then placed in labelled plastic bags and stored in a dark box being removed only during the filling of the feeding units. This was done to prevent the light from affecting the cod liver oil. No diet which had been mixed more than 14 days was fed in the experiments.

(b) The Experimental Diets.

Experiment I: A Preliminary Study of the Factors Affecting the Nutritive.

In the 14-day pre-experimental period the chickens were fed a pre-experimental diet which had the following composition and contained 9.31% protein.

The Pre-Experimental Diet

Wheat	65.0
Whey Powder	6.0
CaCO ₃	1.0
NaCl	0.5
Cod Liver Oil	1.0
Sugar	26.5
	<hr/>
	100.0
	<hr/>

The composition of the basal diet used in the experimental diets, to which the meatmeals were added in place of some of the sugar to formulate the experimental diets, is given below:

The Basal Diet

Wheat	65.0
Whey Powder	6.0
CaCO ₃	1.0
NaCl	0.5
Cod Liver Oil	1.0
	<hr/>
	73.5
	<hr/>
Supplementary protein)	26.5
Sugar)	
	<hr/>
	100.0
	<hr/>

The basal diet formed 73.5 parts of the experimental diet and contributed to 9.31% of the protein in the experimental diet.

T A B L E VI.

The Experimental Diets Fed in Experiment I.

	Longburn			Wanganui			Gisborne			Waitaki		
	11%	15%	20%	11%	15%	20%	11%	15%	20%	11%	15%	20%
Basal diet	73.500	73.500	73.500	73.500	73.500	73.500	73.500	73.500	73.500	73.500	73.500	73.500
Meatmeal	2.754	9.287	17.460	2.239	7.537	14.170	2.805	9.443	17.740	3.339	11.240	21.110
Sugar	23.746	17.213	9.040	24.261	18.963	12.330	23.695	17.057	8.760	23.161	15.260	5.390
	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000
	Pareora			Belfast			Fairfield			Waitara		
	11%	15%	20%	11%	15%	20%	11%	15%	20%	11%	15%	20%
Basal diet	73.500	73.500	73.500	73.500	73.500	73.500	73.500	73.500	73.500	73.500	73.500	73.500
Meatmeal	2.879	9.694	18.210	3.101	10.440	19.610	2.786	9.378	17.620	2.658	8.945	16.810
Sugar	23.621	16.806	8.290	23.399	16.060	6.890	23.714	17.122	8.880	23.842	17.555	9.690
	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000
	Patea			Makarewa			Gear					
	11%	15%	20%	11%	15%	20%	11%	15%	20%	11%	15%	20%
Basal diet	73.500	73.500	73.500	73.500	73.500	73.500	73.500	73.500	73.500			
Meatmeal	2.805	9.443	17.740	2.879	9.694	18.220	2.724	9.170	17.230			
Sugar	23.695	17.057	8.760	23.621	16.806	8.280	23.776	17.330	9.270			
	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000			

Experiment II: To Study the Influence of the Varying Levels of Calcium and Phosphorus on the Growth Rate of the Chicken.

The pre-experimental diet was the same as that used in Experiment I, but the basal diet to which the meat and the various calcium salts were added had the following composition:

<u>The Basal Diet</u>		
Wheat	65.0	
Whey Powder	6.0	
Cod Liver Oil	1.0	
NaCl	0.5	
	<hr/>	
	72.5	
	<hr/>	
Meat protein	}	27.5
Calcium salts		
Sugar		
		<hr/>
		100.0
		<hr/>

The basal diet which forms 72.5 parts of the experimental diet also contributes to 9.31% of the protein, 0.042% of the calcium, and 0.247% of the phosphorus in the experimental diet.

T A B L E VII.

The Experimental Diets Fed in Experiment II.

A. Diets containing 20% Protein and 0.8% Phosphorus with Varying Calcium Levels.

	Ca 0.5%	Ca 0.9%	Ca 1.4%	Ca 1.9%
Basal diet	72.500	72.500	72.500	72.500
Meat	15.300	15.300	15.300	15.300
Sugar	9.743	8.743	8.336	7.086
CaCO ₃	0.282	1.282	1.254	2.504
Ca ₃ (PO ₄) ₂			1.940	1.940
Ca(H ₂ PO ₄) ₂ ·H ₂ O	2.175	2.175	0.670	0.670
	100.000	100.000	100.000	100.000

B. Diets containing 20% Protein and 0.3% Phosphorus with Varying Calcium Levels.

	Ca 0.5%	Ca 0.9%	Ca 1.4%
Basal diet	72.500	72.500	72.500
Meat	15.300	15.300	15.300
Sugar	11.091	10.091	8.841
CaCO ₃	0.845	1.845	3.095
Ca ₃ (PO ₄) ₂	0.264	0.264	0.264
	100.000	100.000	100.000

Experiment III:

A Study of the Effect of Heat on the Nutritive Value and "Available Lysine" of Animal Protein.

In this experiment the pre-experimental diet and the basal diet were the same as that used in Experiment I.

T A B L E VIII.

The Experimental Diets Fed in Experiment II.

	Freeze Dried Meat			Autoclaved 5 mins. at 240°F.		
	11%	15%	20%	11%	15%	20%
Basal diet	73.500	73.500	73.500	73.500	73.500	73.500
Protein	2.770	9.330	17.530	2.380	8.013	15.060
Sugar	23.730	17.170	8.970	24.120	18.487	11.440
	100.000	100.000	100.000	100.000	100.000	100.000
	Autoclaved 25 mins. at 240°F.			Autoclaved 2 hrs. at 240°F.		
	11%	15%	20%	11%	15%	20%
Basal diet	73.500	73.500	73.500	73.500	73.500	73.500
Protein	2.414	8.128	15.280	2.414	8.128	15.280
Sugar	24.086	18.372	11.220	24.086	18.372	11.220
	100.000	100.000	100.000	100.000	100.000	100.000
	Autoclaved 2 hrs. at 270°F.					
	11%	15%	20%			
Basal diet	73.500	73.500	73.500			
Protein	2.370	7.980	15.000			
Sugar	24.130	18.520	11.500			
	100.000	100.000	100.000			

Experiment IV: The Supplementation of a Meatmeal with L-lysine.

The pre-experimental diet was the same as that used in Experiment I, but the basal diet to which the meatmeal was added contained only 3.88% protein. This synthetic basal diet was similar to the standard basal diet used in Experiment I, except that it contained no wheat or whey powder which were replaced by starch since these contributed to the protein in the standard basal diet. The synthetic basal diet was deficient in Vitamin E and Vitamin B complex and essential minerals due to the removal of the wheat and whey powder. The Vitamin E was replaced by the addition of wheat germ oil, while the Vitamin B complex was replaced by the addition of a yeast extract in the form of a special vegemite starch mixture (1:4). The minerals were replaced by a special mineral mixture (Kratzer, et al. 1958).

The Composition of the Synthetic Basal Diet

Starch	37.5
Starch-Vegemite Mixture	12.5
Wheat germ oil	1.0
Mineral Mixture	2.0
Cod liver oil	1.5
CaCO ₃	1.0
	<hr/>
	55.5
	<hr/>
protein)	
sugar)	44.5
lysine)	<hr/>
	100.0
	<hr/>

The synthetic basal diet supplied 55.5 parts of the experimental diets and contributed to 3.88% of the protein in the experimental diets.

TABLE IX.

The Experimental Diets Fed in Experiment IV.

	Meatmeal		Meatmeal supplemented with Lysine	
	15%	20%	15%	20%
Basal diet	15.50	56.50	56.500	56.500
Meatmeal	20.40	29.60	20.400	29.600
Sugar	23.10	13.90	22.753	13.398
L-lysine	-	-	0.347	0.502
	<u>100.00</u>	<u>100.00</u>	<u>100.00</u>	<u>100.00</u>

Experiment V: To Study the Protein Quality and the "Available Lysine" in N.Z. Meatmeals.

In the 14-day pre-experimental period the chickens were fed a diet which contained 8% of protein and had the following composition:

The Pre-Experimental Diet.

Millers offals	10.00
Barley	11.50
Maize	10.00
Oats	10.00
Whey Powder	10.00
Yeast	3.00
Cod liver oil	1.00
NaCl	0.50
Ca ₃ (PO ₄) ₂	2.68
Sugar	41.32
	<hr/>
	100.00
	<hr/>

The diet was designed to provide 0.8% phosphorus and 1.1% calcium, as well as an adequate level of vitamins and minerals.

The basal ration to which the meatmeals and casein were added as sources of supplementary protein to formulate the experimental ration was the same as the pre-experimental diet used.

Basal Diet

Millers offals	10.0	
Barley	11.5	
Maize	10.0	
Oats	10.0	
Whey Powder	10.0	
Yeast	3.0	
Cod liver oil	1.0	
NaCl	0.5	
	<hr/>	
	56.0	
	<hr/>	
Meatmeal	}	44.0
Calcium salts		
Sugar		
		<hr/>
		100.0
		<hr/>

TABLE X.

The Experimental Diets Fed in Experiment V.

	Basal Diet (Negative Control)	Casein	Waitara
Basal diet	56.00	56.00	56.00
Meatmeal	-	3.53	4.71
$\text{Ca}_3(\text{PO}_4)_2$	2.68	2.58	1.91
CaCO_3	-	0.10	0.25
Sugar	41.32	37.79	37.13
	100.00	100.00	100.00
	Gisborne	Wanganui	Patea
Basal diet	56.00	56.00	56.00
Meatmeal	4.98	3.98	4.98
$\text{Ca}_3(\text{PO}_4)_2$	2.14	2.67	2.14
CaCO_3	-	-	0.05
Sugar	36.88	37.35	36.83
	100.00	100.00	100.00
	Fairfield	Belfast	Makarewa
Basal diet	56.00	56.00	56.00
Meatmeal	4.94	3.53	5.10
$\text{Ca}_3(\text{PO}_4)_2$	1.50	-	1.81
$\text{CaH PO}_4 \cdot 2\text{H}_2\text{O}$	0.37	2.37	-
CaCO_3	-	0.07	0.12
Sugar	37.19	38.03	36.97
	100.00	100.00	100.00

CHAPTER III

RESULTS

The Meatmeals.

The results of the analysis of the eleven meatmeals studied in Experiment I are given in Table XI.

TABLE XI

The Analysis of the Meatmeals.

	Ash %	Ca %	P %	Protein %	Fat %	Moisture %
Longburn	20.80	8.12	3.58	61.25	10.75	6.55
Gear	4.77	0.80	0.30	62.04	23.80	7.35
Waitaki	32.30	12.68	5.29	50.60	11.26	6.34
Pareora	19.67	7.06	3.66	58.70	9.20	7.42
Belfast	24.20	8.60	2.03	54.50	12.75	4.70
Fairfield	21.86	8.10	3.45	60.67	10.13	5.50
Gisborne	12.88	2.79	1.20	60.25	18.18	7.70
Wanganui	3.32	0.56	0.11	75.50	15.22	5.43
Patea	16.21	4.24	2.25	60.25	11.90	7.63
Makarewa	19.17	6.07	3.46	58.70	14.40	6.71
Waitara	19.82	4.60	3.37	63.60	13.85	5.50
Mean \pm S.E.	17.73 \pm 2.52	5.78 \pm 1.10	2.61 \pm 0.48	60.55 \pm 1.85	13.77 \pm 1.26	6.44 \pm 0.31

The data given in Table XI shows that N.Z. Meatmeals are heterogenous products and the range of values obtained for ash, protein, fat and moisture are shown in Table XII.

T A B L E X I I

The Range of Values for Ash, Protein, Fat and Moisture
Obtained in the Analysis of the Meatmeals.

Ash	32.30%	3.22%
Protein	75.50%	50.60%
Fat	23.80%	9.20%
Moisture	7.63%	4.70%

The meatmeals which were found to be low in protein were also shown to be high in either ash or fat, and in some cases both the ash and fat constituted a high percentage of the meatmeal. The high ash content of some of the meatmeals was due to the fact that the materials used in the processing of these meatmeals contained a high percentage of bone, while the meatmeals which had a high fat content were those which had been manufactured by processes where the extraction of fat from the digested material had not been completed. The moisture content of the meatmeals was low and, therefore, any variations in the moisture content do not influence the level of protein found in the meatmeals.

The calcium and phosphorus content of the meatmeals represented about 50% of the mineral content of the ash, and when the calcium and phosphorus content of the meatmeals were correlated with the ash present in the meatmeals, correlations of 0.96 and 0.91 respectively were found to exist. (see Appendix XX). Therefore, the level of calcium and phosphorus in the meatmeals could be estimated from the ash content using the regression equation

= $0.423 X - 1.72$ for calcium, and the regression equation = $0.173 X - 0.46$
for phosphorus.

Experiment 1: A Preliminary Study of the Factors Affecting the Nutritive Value of N. Z. Meatmeals.

The eleven meatmeals were studied in two trials with Longburn as a standard in each because the facilities available did not make it possible to test the eleven meatmeals together. When Longburn (Trial I) was compared to Longburn (Trial II) the two regression lines were found to be parallel with no significant difference between the elevation of the two regression lines. The analysis of covariance is shown in Table XIII, while the data is presented in Appendices II and IV.

T A B L E XIII.

The Analysis of Covariance.
Longburn (Trial I); Longburn (Trial II)

	d.f.	$\sum x^2$	$\sum xy$	$\sum y^2$	Reg. Coeff.	Deviations from Regression		
						d.f.	$\sum d.yx^2$	Mean square
Longburn Trial I	26	0.78374	0.77696	0.93066	0.99	25	0.16042	0.0064
Longburn Trial II	29	0.81659	0.74184	1.00835	0.91	28	0.3344	0.0119
Within						53	0.4948	0.0093
Regression Coefficient						1	0.0028	0.0028
Common	55	1.60033	1,51880	1.93901	0.95	54	0.4976	0.0092
Adjusted Means						1	0.0000	0.0000
Total	56	1.60179	1.52036	1.94065		55	0.4976	

The difference between regression coefficients:

$$F = \frac{0.0028}{0.0093} = \text{less 1 for } \frac{1}{55} \text{ d.f. required } F \begin{matrix} (4.02) \\ (7.12) \end{matrix} \text{ not significant}$$

The difference in elevation:

$$F = \frac{0.0010}{0.0092} = \text{less 1 for } \frac{1}{56} \text{ d.f. required } F \begin{matrix} (4.02) \\ (7.11) \end{matrix} \text{ not significant}$$

These results show that the data obtained from the two trials was not greatly influenced by external factors operating in one trial and not the other.

Trial I.

The analysis of covariance for Trial I is given in Table XIV, while the data is presented in Appendix II.

T A B L E X I V .
Analysis of Covariance
 Trial I Meatmeals

	d.f.	$\sum x^2$	$\sum xy$	$\sum y^2$	Reg. Coeff.	Deviations from Regression		
						d.f.	$\sum dyx^2$	Mean square
Longburn	26	0.78374	0.77696	0.93066	0.99	25	0.16042	0.0064
Gear	26	1.00597	1.18582	1.63418	1.18	25	0.2364	0.0095
Waitaki	26	0.37662	0.44466	0.72513	1.18	25	0.2001	0.0080
Pareora	26	0.91472	1.23430	2.20103	1.35	25	0.5355	0.0214
Belfast	26	0.94085	1.24363	1.81891	1.32	25	0.1751	0.0070
Fairfield	26	0.87623	1.15464	1.81012	1.32	25	0.2886	0.0115
Within						150	1.5961	0.0106
Regression Coefficient						5	0.0758	0.0151
Common	156	4.89813	6.04001	9.12003	1.23	155	1.6719	

The difference between regression coefficients:

$$F = \frac{0.0151}{0.0106} = 1.42 \text{ for } \frac{5}{150} \text{ d.f. required } F \begin{matrix} (2.27) \\ (3.14) \end{matrix} \text{ not significant}$$

The analysis of the regression lines showed that there were no significant differences between the regression coefficients and, therefore, all the meatmeals tested in Trial I can be said to have been drawn from the same population.

Trial II.

The analysis of covariance for Trial II is given in Table XV, and the data is presented in Appendix II.

COMPARISON OF NUTRITIVE VALUES OF PROTEIN
IN MEAT MEALS.

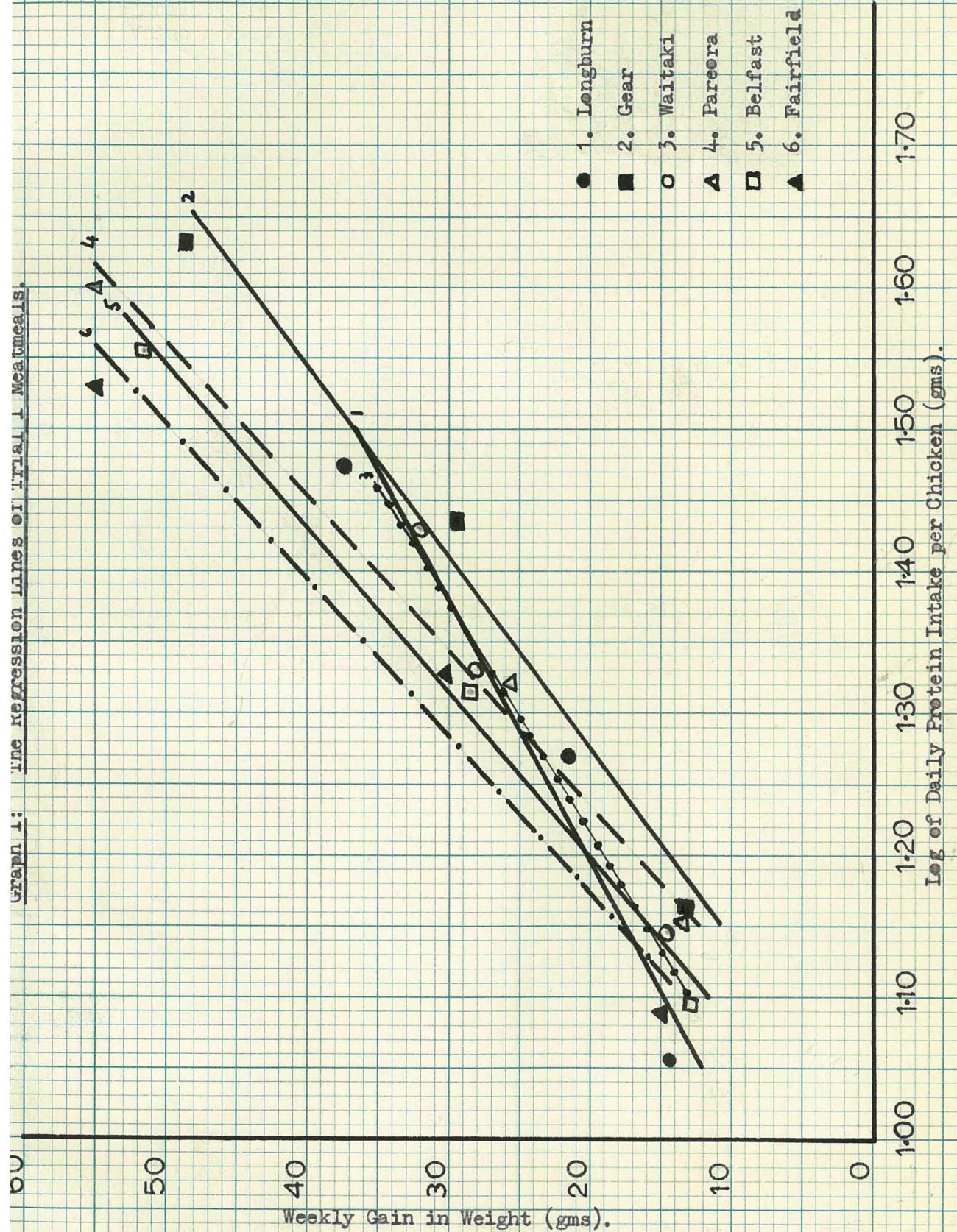
Meat Meal No.	Available Lysine Value g lysine/16g N.	Relative Nutritive Value from Bioassay and 95% fiducial limits.
1	4.8	100
8	4.6	101 (87.8 - 117.3)
6	4.8	108.5 (92.2 - 121.0)
7	4.8	99.0 (85.9 - 114.1)
11	4.9	Bioassay not possible*
4	5.3	82.2 (73.8 - 91.6)
5	5.5	100.4 (90.2 - 111.8)
9	5.6	104.2 (90.5 - 119.9)
3	5.7	94.4 (84.9 - 105.0)
2	5.9	91.7 (81.9 - 101.6)
10	6.5	Bioassay not possible*

Bioassays 4 2 3 7 1 5 8 9 6

* slopes significantly > standard.

Adapted from N.D.Grace & E.L.Richards 1964 J.Sci.Fd.Agric. 15, 711

Graph 1: The Regression Lines of Final 1 Meatmeals.



T A B L E XV.

Analysis of Covariance
Trial II Meatmeals

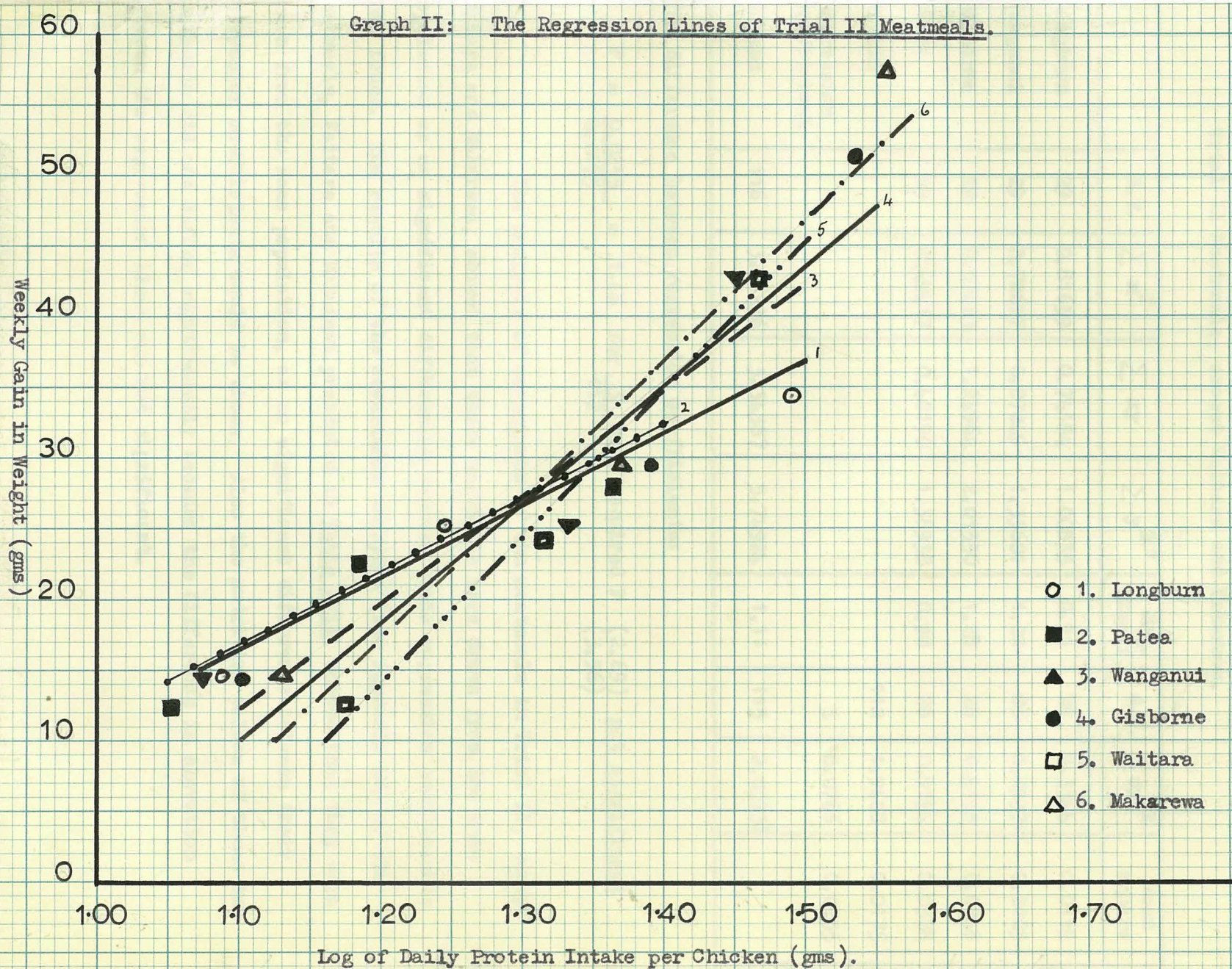
	d.f.	$\sum x^2$	$\sum xy$	$\sum y^2$	Reg. Coeff.	Deviations from Regression		
						d.f.	$\sum dyx^2$	Mean square
Longburn	29	0.81659	0.74184	1.00835	0.91	28	0.3344	0.0119
Wanganui	29	0.72697	0.87049	1.33601	1.20	28	0.2937	0.0105
Gisborne	29	0.97463	1.24964	2.01550	1.28	28	0.4133	0.0148
Waitara	29	0.43926	0.78347	1.78786	1.78	28	0.3904	0.0139
Patea	29	0.49417	0.48190	1.38938	0.98	28	0.9194	0.0328
Makarewa	29	0.88469	1.28829	2.19983	1.46	28	0.3238	0.0116
Within						168	2.6750	0.0159
Regression Coefficient						5	0.2983	0.0596
Common	174	4.33631	5.41563	9.73693	1.25	173	2.9733	

The difference between regression coefficients:

$$F = \frac{0.0596}{0.0159} = 3.75 \text{ for } \frac{5}{168} \text{ d.f. required } F \begin{matrix} (2.27) \\ (3.12) \end{matrix} \text{ significant difference^4}$$

The results from this analysis of covariance showed that one or more of the meatmeals tested differs significantly from the rest of the meatmeals. This group of meatmeals, except for Waitara and Makarewa, were again analysed and the analysis of covariance is shown in Table XVI.

Graph II: The Regression Lines of Trial II Meatmeals.



T A B L E XVI.

The Analysis of Covariance

Trial II Meatmeals, except Waitara and Makarewa.

	d.f.	$\sum x^2$	$\sum xy$	$\sum y^2$	Reg. Coeff.	d.f.	$\sum dyx^2$	Mean square
Longburn	29	0.81659	0.74184	1.00835	0.91	28	0.3344	0.0119
Wanganui	29	0.72697	0.87049	1.33601	1.20	28	0.2937	0.0105
Gisborne	29	0.97463	1.24964	2.01550	1.28	28	0.4133	0.0148
Patea	29	0.49417	0.48190	1.38938	0.98	28	0.9194	0.0328
Within						112	1.9608	0.0175
Regression Coefficient						3	0.0766	0.0255
Common	116	3.01236	3.34387	5.74924	1.11	115	2.03738	

The difference between regression coefficients:

$$F = \frac{0.0255}{0.0175} = 1.46 \text{ for } \frac{3}{112} \text{ d.f. required } F \begin{matrix} (2.69) \\ (3.96) \end{matrix} \text{ not significant}$$

The analysis of covariance of the meatmeals in Trial II, except Waitara and Makarewa, showed that these meatmeals were drawn from the same population as no significant differences between their regression coefficients were found. The results of the covariance analysis (see Appendix IV) of the meatmeals, Waitara and Makarewa presented in Table XVII, showed that they were drawn from the same population, as no significant difference between their regression coefficients could be shown. Also the nutritive values of these two meatmeals did not differ significantly because the difference in elevation between the regression lines was not significant.

T A B L E XVII.

The Analysis of Covariance

Waitara and Makarewa

	d.f.	$\sum x^2$	$\sum xy$	$\sum y^2$	Reg. Coeff.	d.f.	$\sum dyx^2$	Mean square
Waitara	29	0.43926	0.78347	1.78786	1.78	28	0.3904	0.0139
Makarewa	29	0.88469	1.28829	2.19983	1.46	28	0.3238	0.0116
Within						56	0.7142	0.0127
Regression Coefficient						1	0.03150	0.0315
Common	58	1.32395	2.07176	3.98769	1.56	57	0.7457	0.0131
Adjusted Means						1	0.0210	0.0210
Total	59	1.34129	2.11807	4.11140		58	0.7667	

The difference between regression coefficients:

$$F = \frac{0.03150}{0.0127} = 2.50 \text{ for } \frac{1}{56} \text{ d.f. required } F \begin{matrix} (4.02) \\ (7.12) \end{matrix} \text{ not significant}$$

The difference in elevation:

$$F = \frac{0.0210}{0.0131} = 1.60 \text{ for } \frac{1}{57} \text{ d.f. required } F \begin{matrix} (4.01) \\ (7.10) \end{matrix} \text{ not significant}$$

The regression lines of Trial I and Trial II are plotted in graphs I and II (see Appendix III), while the regression coefficients, together with their fiducial limits (see Appendix V), are presented in Table XVIII.

T A B L E XVIII.

The Regression Coefficients of the Meatmeals.

Trial I			
Longburn	0.99	±	0.08
Gear	1.18	±	0.08
Waitaki	1.18	±	0.08
Pareora	1.35	±	0.13
Belfast	1.32	±	0.08
Fairfield	1.32	±	0.10
Trial II			
Longburn	0.91	±	0.10
Patea	0.97	±	0.18
Wanganui	1.20	±	0.09
Gisborne	1.28	±	0.10
*Makarewa	1.46	±	0.09
*Waitara	1.78	±	0.10

An examination of Table XVIII shows that the fiducial limits of the regression coefficients of each meatmeal does not vary greatly. Also the meatmeals Makarewa and Waitara, which were found to belong to a different population, have the highest regression coefficients.

To examine whether any significant differences between the nutritive value of the meatmeals (except Waitara and Makarewa) existed, the nutritive value of each meatmeal compared was expressed in terms of relative potency which was calculated (see Appendix VI) using Longburn as the standard, and these relative potencies, together with their 95% fiducial limits (see Appendix VII), are presented in Table XIX. Waitara and Makarewa were not included in these comparisons because they formed a second group whose nutritive values had already been compared in Table XVII.

T A B L E XIX.

The Relative Potencies of the Meatmeals.

Trial I			
Longburn (standard)		100.0	
Waitaki	84.9	94.4	105.0
Pareora	81.9	91.7	101.6
Belfast	90.2	100.4	111.8
Fairfield	92.2	108.5	121.0
Gear	73.8	82.2	91.6
Trial II			
Longburn (standard)		100.0	
Wanganui	90.5	104.2	119.9
Gisborne	85.9	99.0	114.1
Patea.	87.8	101.5	117.3

The results of the comparisons made (see Appendix VIII) between the various meatmeals are presented in Table XX. These results show that the nutritive value of the meatmeals was not uniform, as significant differences in the nutritive values were found when the various meatmeals were compared. These comparisons are presented in Table XX.

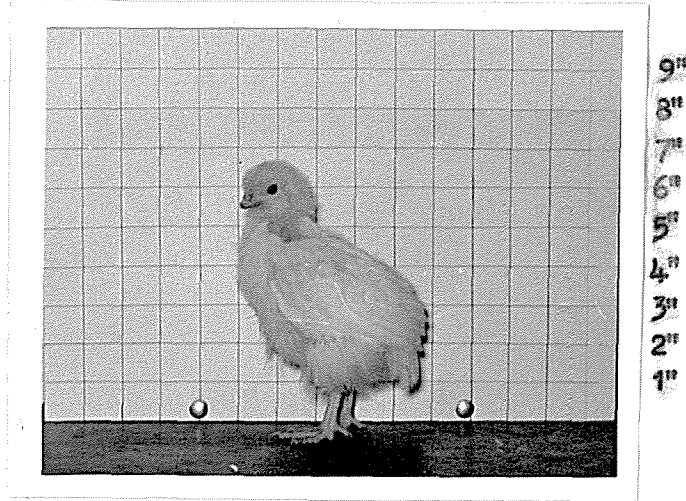
T A B L E XX.

Comparisons between the Nutritive Values of the Meatmeals.

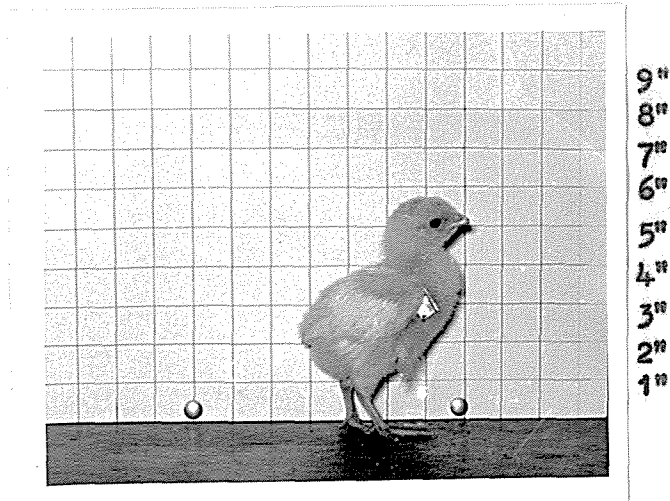
Trial I.			
Fairfield	v	Gear	*
Fairfield	v	Pareora	*
Fairfield	v	Waitaki	*
Gear	v	Belfast	*
Gear	v	Pareora	N.S.
Belfast	v	Pareora	N.S.
Trial II.			
Wanganui	v	Patea	N.S.

Fig. 6:

The Influence of the Varying Levels of Protein
on the Growth Rate of the Chicken.



(a) 20% protein.



(b) 11% protein.

Experiment II: To Study the Influence of the Varying Levels of Calcium and Phosphorus on the Growth Rate of the Chicken.

The experimental diets used in Experiment I contained varying levels of calcium and phosphorus in the ratio of 2:1, and Table XXI gives the calcium and phosphorus content of the experimental diets fed.

T A B L E XXI.

The Calcium and Phosphorus Content of the Experimental Diets Fed in Experiment I.

Level of Protein Ca and P expressed as % of diet	11%		15%		20%	
	Ca	P	Ca	P	Ca	P
Longburn	0.62	0.35	1.12	0.58	1.80	0.87
Gear	0.42	0.26	0.47	0.28	0.66	0.32
Waitaki	0.82	0.43	1.88	0.85	3.10	1.37
Pareora	0.60	0.36	1.08	0.61	1.71	0.92
Fairfield	0.63	0.35	1.16	0.57	1.82	0.86
Wanganui	0.41	0.26	0.44	0.26	0.50	0.27
Gisborne	0.48	0.29	0.66	0.37	0.90	0.47
Waitara	0.52	0.34	0.79	0.55	1.20	0.82
Patea	0.52	0.32	0.79	0.46	1.20	0.75
Makarewa	0.57	0.36	0.99	0.59	1.50	0.88
Belfast	0.67	0.27	1.25	0.46	1.90	0.75

An examination of the table showed that the diets varied widely in their levels of calcium and phosphorus, and Experiment II was designed to measure the influence of these differences on the growth rate of the chicken.

An F-test of the feed intakes (see Appendix XI) of the different experimental groups showed that the feed intakes did not differ significantly. Therefore the variation in feed intakes of the experimental groups would have no influence on the results obtained by the various treatments. The F-test for the feed intakes is presented in Table XXII.

T A B L E XXII.

The F-test for the Feed Intakes

Source of Variation	SS	d.f.	Mean square	F	Required F	Result
Treatment	3,386.6	6	564.4	3.60	4.21 8.26	N.S.
Residual	<u>1,098.8</u>	7	157.0			
Total	4,485.4	13				

The results of the treatments involving 3 levels of calcium (0.5%, 0.9% and 1.4%), and 2 levels of phosphorus (0.3% and 0.8%) were statistically analysed and an analysis of these results is shown in Table XXIII. The data is presented in Appendix IX.

T A B L E XXIII.

An Analysis of Variance of the Weight Gain Measured on Diets containing

0.3%, 0.8% Phosphorus, and 0.5%, 0.9%, 1.4% Calcium.

Source of Variation	SS	d.f.	Mean square	F	Required F	Result
Blocks	17.79	1	17.79	0.27	6.61 16.26	N.S.
Calcium	1,158.41	2	579.21			
Phosphorus	1,472.97	1	1472.97			
Calcium x Phosphorus	1,028.27	2	514.13	8.47	5.79 13.27	*
Error	303.59	5	60.72			
Total	3,981.03	11				

A significant difference at the 5% level was found with the calcium phosphorus interaction.

The F-ratios of calcium and phosphorus were not tested because mean square of the calcium phosphorus interaction was high. This is best explained in terms of the following equations.

$$\begin{aligned} \text{Calcium M.S.} &= b^2 + b^2 \text{ Ca} + b^2 \text{ Ca} \times \text{P} \\ \text{Phosphorus M.S.} &= b^2 + b^2 \text{ P} + b^2 \text{ Ca} \times \text{P} \\ \text{Calcium} \times \text{Phosphorus M.S.} &= b^2 + b^2 \text{ Ca} \times \text{P} \end{aligned}$$

An examination of Table XXIII shows that the calcium x phosphorus M.S. contributes to all the variance of the calcium M.S., and half the variance of the phosphorus M.S. This means that the calcium x phosphorus interaction is the most important source of variation. For this reason also, the pooled means were not examined in the table of means presented below.

T A B L E XXIV.
The Table of Means.

Phosphorus	0.5%	0.9%	1.4%	Phosphorus Means
0.3%	125.31	109.59	79.34	104.74
0.8%	127.69	126.79	126.23	126.90
Calcium Means	126.50	118.19	102.78	

d (.05) for 5 d.f. = 2.571

d (.01) for 5 d.f. = 4.032

calcium x phosphorus means

$$d (.05) = 2.571 \sqrt{\frac{2 \times 60.72}{2}} = 20.03$$

$$d (.01) = 4.032 \sqrt{\frac{2 \times 60.72}{2}} = 31.41$$

The results from the table of means show that:-

(a) Within the calcium levels:

- 0.5 Ca - no difference between 0.8 and 0.3 phosphorus
- 0.9 Ca - no difference between 0.8 and 0.3 phosphorus
- 1.4 Ca - 0.8 > 0.3

Within the 1.4% level of calcium the higher level of phosphorus gave the best growth rate for the chicken. No significant differences between the 0.3% and the 0.8% phosphorus were found on the diets containing 0.5% and 0.9% calcium.

(b) Within the phosphorus levels:

- 0.3 P - 0.5 0.9 > 1.4
- 0.8 P - no significant difference

Within the 0.3% phosphorus level the increase in the level of calcium to 1.4% depressed the growth rate of the chicken. No difference in growth rate was found between the calcium levels of 0.5%, 0.9% and 1.4% on diets containing 0.8% phosphorus.

In the second part of the experiment, in which the calcium levels were varied from 0.5% to 1.9% in diets containing 0.8% phosphorus, no significant difference in the growth rate of the chicken was found. The analysis of variance of these results is presented in Table XXV, while the data is shown in Appendix X.

TABLE XXV.

An Analysis of Variance of the Varying Calcium Levels (0.5%, 0.9%, 1.4% and 1.9%) in Diets Containing 0.8% Phosphorus.

Source of Variation	SS	d. f.	Mean square	F	Required F	Result
Level	22.12	3	7.37	less 1	9.28 29.46	N. S.
Run	15.42	1	15.42	less 1	10.13 34.12	N. S.
Error	76.75	3	25.58			
Total	114.29	7				

The experimental diets used in Experiment I which were low in calcium and phosphorus contained 0.5% calcium and 0.3% phosphorus, while the diets high in calcium and phosphorus contained 1.9% calcium and 0.8% phosphorus. A comparison between the 0.3% phosphorus, 0.5% calcium diet, and the 0.8% phosphorus, 1.4% calcium diet showed no significant difference; while a further comparison between the 0.8% phosphorus, 1.4% calcium diet, and 0.8% phosphorus, 1.9% calcium diet also showed no significant difference in the growth rate of the chicken. Therefore, under the conditions of Experiment I, the varying levels of calcium and phosphorus in the experimental diets, with the exception of Waitaki, did not influence the growth rate of the chicken. Since the experimental diet (20% protein) which was formulated from the Waitaki meatmeal contained 3.10% calcium and 1.37% phosphorus, it is probable that this high level of calcium could have influenced the growth rate of the chickens when they were fed this diet.

Experiment III: A Study of the Effect of Heat on the Nutritive Value and their "Available Lysine" of Animal Protein.

A. The Analysis of the Meat Samples.

Table XXVI shows the analysis of the autoclaved meat samples for moisture fat, protein and ash.

T A B L E XXVI.

The Analysis of the Meat Samples.

	Moisture %	Fat %	Protein %	Ash %
Freeze Dried Meat	12.40	27.90	61.00	2.97
5 mins. at 240°F	11.60	18.90	71.00	2.77
25 mins. at 240°F	11.40	17.90	70.00	2.87
2 hrs. at 240°F	11.70	16.60	70.00	2.36
2 hrs. at 270°F	10.45	17.15	71.30	2.31

The autoclaved meat samples showed only a small difference in moisture, fat and protein content when compared with each other, while the freeze dried meat sample was found to be higher in fat and lower in protein when compared with the autoclaved samples. The difference between the freeze dried meat sample and the autoclaved meat sample can be explained by the fact that some of the fat was lost during the autoclaving of the meat samples.

B. The "Available Lysine".

The "available lysine" of the freeze dried and the autoclaved meat samples is shown in Table XXVII.

T A B L E XXVII.

The "Available Lysine" of the Meat Samples.

Treatment	Available $\frac{\text{gm lysine}}{16 \text{ gm N}}$
(1) Freeze Dried Meat	9.95
(2) 5 mins. @ 240°F.	9.90
(3) 25mins. @ 240°F.	9.86
(4) 2 hrs. @ 240°F.	9.35
(5) 2 hrs. @ 270°F.	8.42

The "available lysine" was reduced by autoclaving the meat for various temperatures and for varying autoclaving intervals. The higher autoclaving temperature greatly accelerated the reduction of the "available lysine" as shown by a comparison between treatment 4 (2 hrs. at 240°F) and treatment 5 (2 hrs. at 270°F). The comparison between the various samples which had been autoclaved for various autoclaving intervals at 240°F showed that there was a progressive reduction in "available lysine" as the autoclaving increased; as demonstrated by the difference between treatment 2 (5 minutes at 240°F) and treatment 3 (25 minutes at 240°F) which showed only a small reduction in "available lysine", and the difference between treatment 2 (5 minutes at 240°F) and treatment 4 (2 hrs. at 240°F) which showed a much greater reduction in "available lysine".

Table XXVIII shows the reduction in "available lysine" when the autoclaved samples were compared with freeze dried meat.

T A B L E XXVIII.

The Reduction in "Available Lysine" when the Autoclaved Meat Samples were Compared to the Freeze Dried Meat.

Treatment	% Reduction in "Available Lysine"
(1) Freeze Dried Meat	-
(2) 5 mins. at 240°F.	0.5
(3) 25 mins. at 240°F.	0.9
(4) 2 hrs. at 240°F.	6.0
(5) 2 hrs. at 270°F.	15.3

Treatment 5 (2 hrs. at 270°F) which is similar to the conditions experienced by the meatmeals when they are processed showed a 15.3% reduction in the "available lysine", when it was compared to the freeze dried meat.

C. The Nutritive Value of the Autoclaved Meats.

The regression lines of the freeze dried and autoclaved samples of meat were statistically analysed in the following tables. The regression lines are plotted in Graph III and the data is presented in Appendices XII and XIII.

T A B L E X X I X .
Analysis of Covariance.

Freeze Dried Meat; Autoclaved Meat (5 minutes at 240°F).

	d.f.	$\sum x^2$	$\sum xy$	$\sum y^2$	Reg. Coeff.	Deviations from Regression		
						d.f.	$\sum dyx^2$	Mean square
Freeze Dried Meat	27	0.59610	0.88955	1.94541	1.49	26	0.61795	0.0237
5 mins. @ 240°F	28	0.82140	0.81375	1.61131	0.99	27	0.80514	0.0298
Within						53	1.4231	0.0268
Regression Coefficient						1	0.0869	0.0869
Common	55	1.41750	1.70330	3.55672	1.20	54	1.5100	0.0279
Adjusted Means						1	0.0512	0.0512
Total	56	1.48227	1.72226	3.56226		55	1.5612	-

The difference between regression coefficients:

$$F = \frac{0.0869}{0.0268} = 3.42 \text{ for } \frac{1}{53} \text{ d.f. required } F \begin{matrix} (4.02) \\ (7.14) \end{matrix} \quad \underline{\text{not significant}}$$

The difference in elevation:

$$F = \frac{0.0512}{0.0279} = 1.84 \text{ for } \frac{1}{54} \text{ d.f. required } F \begin{matrix} (4.02) \\ (7.13) \end{matrix} \quad \underline{\text{not significant}}$$

T A B L E X X X .
Analysis of Covariance.

Freeze Dried Meat; Autoclaved Meat (25 minutes at 240°F).

	d.f.	$\sum x^2$	$\sum xy$	$\sum y^2$	Reg. Coeff.	Deviations from Regression		
						d.f.	$\sum dyx^2$	Mean square
Freeze Dried Meat	27	0.59610	0.88955	1.94541	1.49	26	0.61795	0.0237
25 mins. @ 240°F.	27	0.93620	1.15930	2.18051	1.23	26	0.74495	0.0286
Within						52	1.36290	0.0262
Regression Coefficient						1	0.02449	0.0245
Common	54	1.53230	2.04885	4.12592	1.34	53	1.38639	0.0262
Adjusted Means						1	0.03064	0.0306
Total	55	1.63060	2.12366	4.18284		54	1.41705	

The difference between regression coefficients:

$$F = \frac{0.0245}{0.0262} = \text{less } 1 \text{ for } \frac{1}{52} \text{ d.f. required } F \begin{matrix} (4.03) \\ (7.15) \end{matrix} \quad \underline{\text{not significant}}$$

The difference in elevation:

$$F = \frac{0.03064}{0.0262} = 1.17 \text{ for } \frac{1}{53} \text{ d.f. required } F \begin{matrix} (4.02) \\ (7.14) \end{matrix} \quad \underline{\text{not significant}}$$

T A B L E XXXI.

Analysis of Covariance.

Freeze Dried Meat; Autoclaved Meat (2 hrs. at 240°F.)

	d.f.	$\sum x^2$	$\sum xy$	$\sum y^2$	Reg. Coeff.	Deviation from Regression		
						d.f.	$\sum dyx^2$	Mean Square
Freeze Dried Meat	27	0.59610	0.88955	1.94541	1.49	26	0.61795	0.0237
2 hrs. @ 240°F	29	0.74181	1.02633	2.03147	1.38	28	0.61149	0.0218
Within						54	1.22944	0.0227
Regression Coefficient						1	0.00391	0.00391
Common	56	1.33791	1.91588	3.97688	1.43	55	1.23335	0.0224
Adjusted Means						1	0.00523	0.00523
Total	57	1.34251	1.92737	4.00560		56	1.23858	

The difference between regression coefficients:

$$F = \frac{0.0039}{0.0227} = \text{less } 1 \text{ for } \frac{1}{54} \text{ d.f. required } F \begin{pmatrix} 4.02 \\ 7.13 \end{pmatrix} \text{ not significant}$$

The difference in elevation:

$$F = \frac{0.00523}{0.0224} = \text{less } 1 \text{ for } \frac{1}{55} \text{ d.f. required } F \begin{pmatrix} 4.02 \\ 7.12 \end{pmatrix} \text{ not significant}$$

T A B L E X X X I I .
Analysis of Covariance.

Freeze Dried Meat; Autoclaved Meat (2 hrs. at 270°F).

	d. f.	$\sum x^2$	$\sum xy$	$\sum y^2$	Reg. Coeff.	Deviation from Regression		
						d. f.	$\sum dyx^2$	Mean square
Freeze Dried Meat	27	0.59610	0.88955	1.94541	1.49	26	0.61795	0.0237
2 hrs. at 270°F.	29	0.72645	1.33708	3.05173	1.84	28	0.59075	0.0211
Within						54	1.20870	0.0224
Regression Coefficient						1	0.03972	0.03972
Common	56	1.32255	2.22663	4.99714	1.68	55	1.24842	0.0227
Adjusted Means						1	0.02144	0.02144
Total	57	1.34352	2.28328	5.15024		56	1.26986	

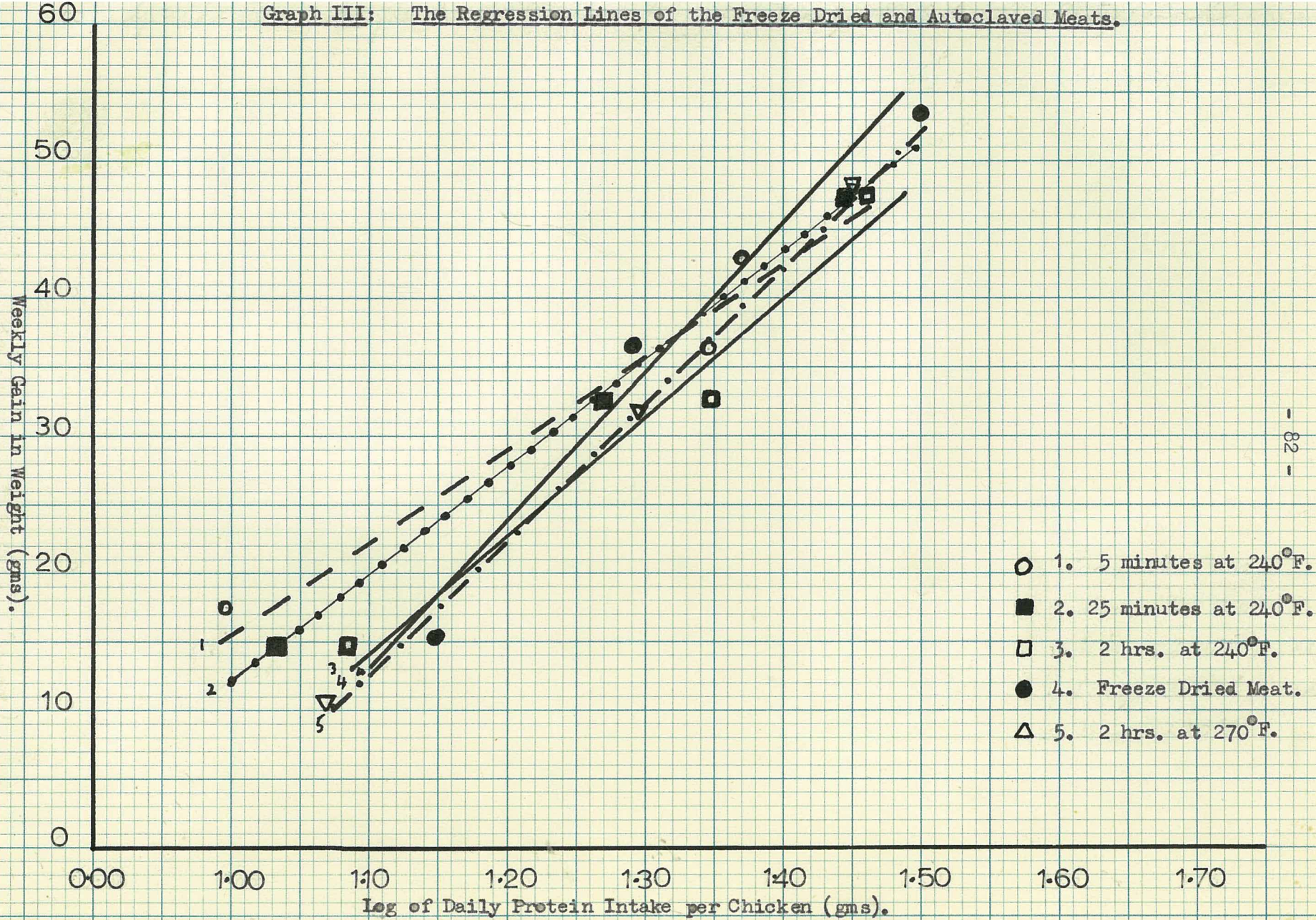
The difference between regression coefficients:

$$F = \frac{0.03972}{0.02238} = 1.78 \text{ for } \frac{1}{54} \text{ d.f. required } F \begin{matrix} (4.02) \\ (7.13) \end{matrix} \quad \underline{\text{not significant}}$$

The difference in elevation:

$$F = \frac{0.02144}{0.02270} = \text{less } 1 \text{ for } \frac{1}{55} \text{ d.f. required } F \begin{matrix} (4.02) \\ (7.12) \end{matrix} \quad \underline{\text{not significant}}$$

Graph III: The Regression Lines of the Freeze Dried and Autoclaved Meats.



In all the comparisons the regression lines of the autoclaved meats when compared with the freeze dried meat were found to be parallel, and the differences between the treatments showed that the nutritive value of the autoclaved samples did not differ significantly from the freeze dried meat; although a progressive decrease in the growth rate was noted when the samples which had been autoclaved for 2 hrs. at 240°F. and 270°F. were compared with the freeze dried meat. The average weekly gain of the freeze dried meat and the autoclaved meat is shown in Table XXXIII.

T A B L E XXXIII.

The Average Weekly Gain of Freeze Dried Meat
Meat Autoclaved for 2 hrs. at 240°F. and for 2 hrs. at 270°F.

Protein Level	11%	15%	20%
Freeze Dried Meat	15.26 gms	36.88 gms	53.59 gms
2 hrs. at 240°F.	14.97 gms	32.73 gms	47.65 gms
2 hrs. at 270°F.	10.05 gms	47.89 gms	47.89 gms

Experiment IV: To Study the Effect of Supplementing Meatmeal with L-lysine.

The regression lines of the meatmeal (see Appendices XVI and XVII) and the lysine supplemented meatmeal, which are plotted in Graph IV, are statistically analysed in Table XXXIV.

T A B L E XXXIV.

Analysis of Covariance.

Meatmeal (control) Meatmeal (lysine supplemented)

	d.f.	$\sum x^2$	$\sum xy$	$\sum y^2$	Reg. Coeff.	Deviation from Regression		
						d.f.	$\sum dyx$	Mean square
Belfast (Control)	18	0.26159	0.34586	0.85446	1.32	17	0.39718	0.02336
Belfast (Lysine)	18	0.27239	0.26543	0.49009	0.97	17	0.23144	0.0136
Within						34	0.62862	0.0185
Regression Coefficient						1	0.01614	0.01614
Common	36	0.53398	0.61129	1.34455	1.14	35	0.64476	0.0184
Adjusted Mean						1	0.03774	
Total	37	0.54540	0.64535	1.44611		36	0.68250	

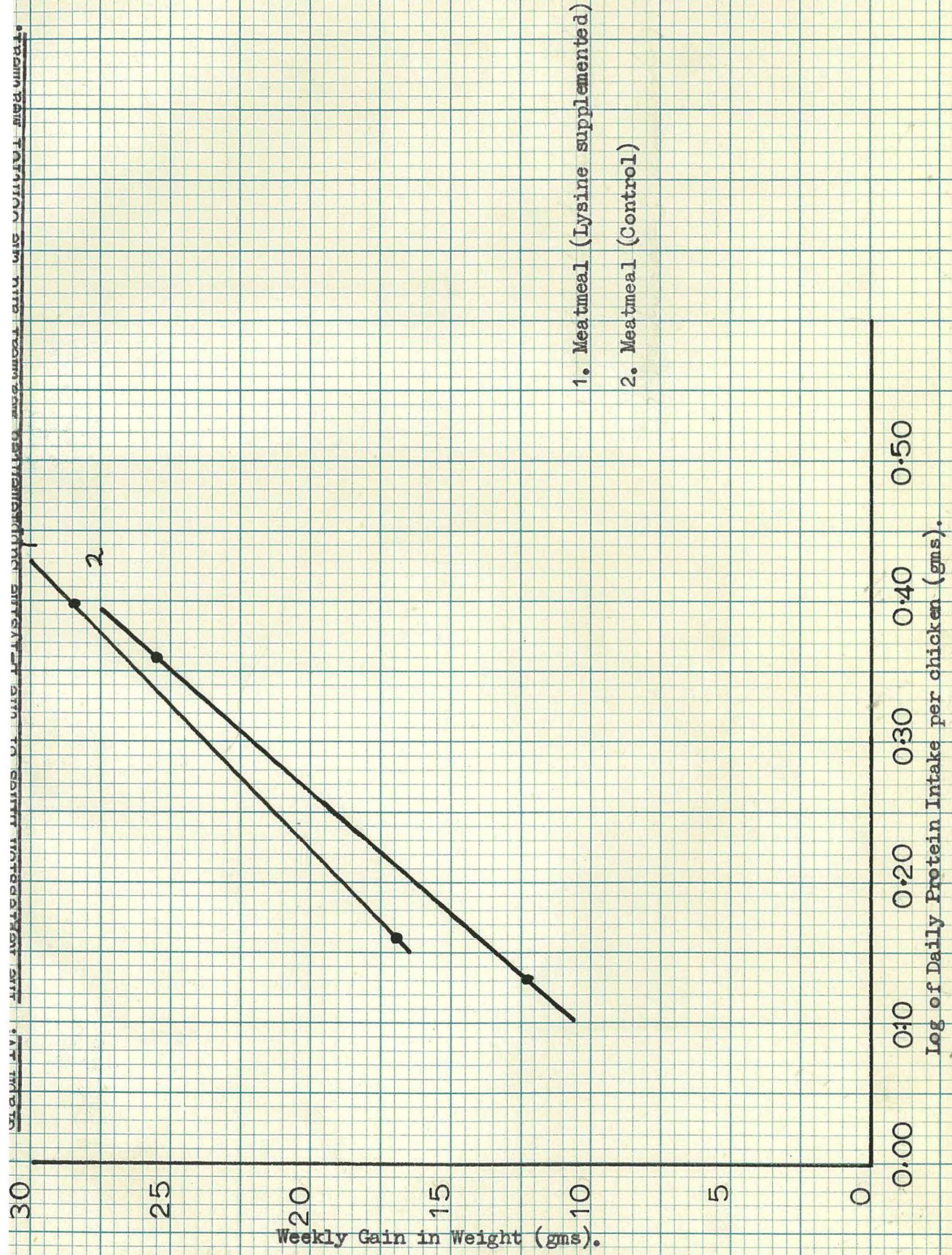
The difference between regression coefficients:

$$F = \frac{0.01614}{0.0185} = \text{less } 1 \text{ for } \frac{1}{34} \text{ d.f. required } F \begin{matrix} (4.13) \\ (7.44) \end{matrix} \text{ not significant}$$

The difference in elevation:

$$F = \frac{0.03774}{0.0184} = 2.05 \text{ for } \frac{1}{35} \text{ d.f. required } F \begin{matrix} (4.12) \\ (7.42) \end{matrix} \text{ not significant}$$

The two regression lines were found to be parallel with no significant difference in elevation between the two regression lines showing that the addition of L-lysine to the meatmeal did not improve its nutritive value.



- 1. Meatmeal (Lysine supplemented)
- 2. Meatmeal (Control)

Experiment V: To Study Protein Quality and the "Available Lysine" in N.Z. Meatmeals.

The gross protein value (see Appendix XVIII) which is a measure of protein quality was determined for the seven meatmeals used in this study, and these values are presented in Table XXXV.

T A B L E XXXV.

The Gross Protein Value and the "Available Lysine" of Meatmeals.

	Gross Protein Value	"Available Lysine" <u>gm lysine</u> 16 gm N
Casein	100	-
Patea	83	4.57
Waitara	93	4.86
Wanganui	98	5.55
Fairfield	98	4.80
Gisborne	99	4.84
Belfast	99	5.51
Makarewa	111	6.50

correlation coefficient 0.84.

Makarewa was found to have the highest gross protein value and Patea the lowest gross protein values, while a large group consisting of Wanganui, Fairfield, Gisborne and Belfast were shown to have intermediate gross protein values.

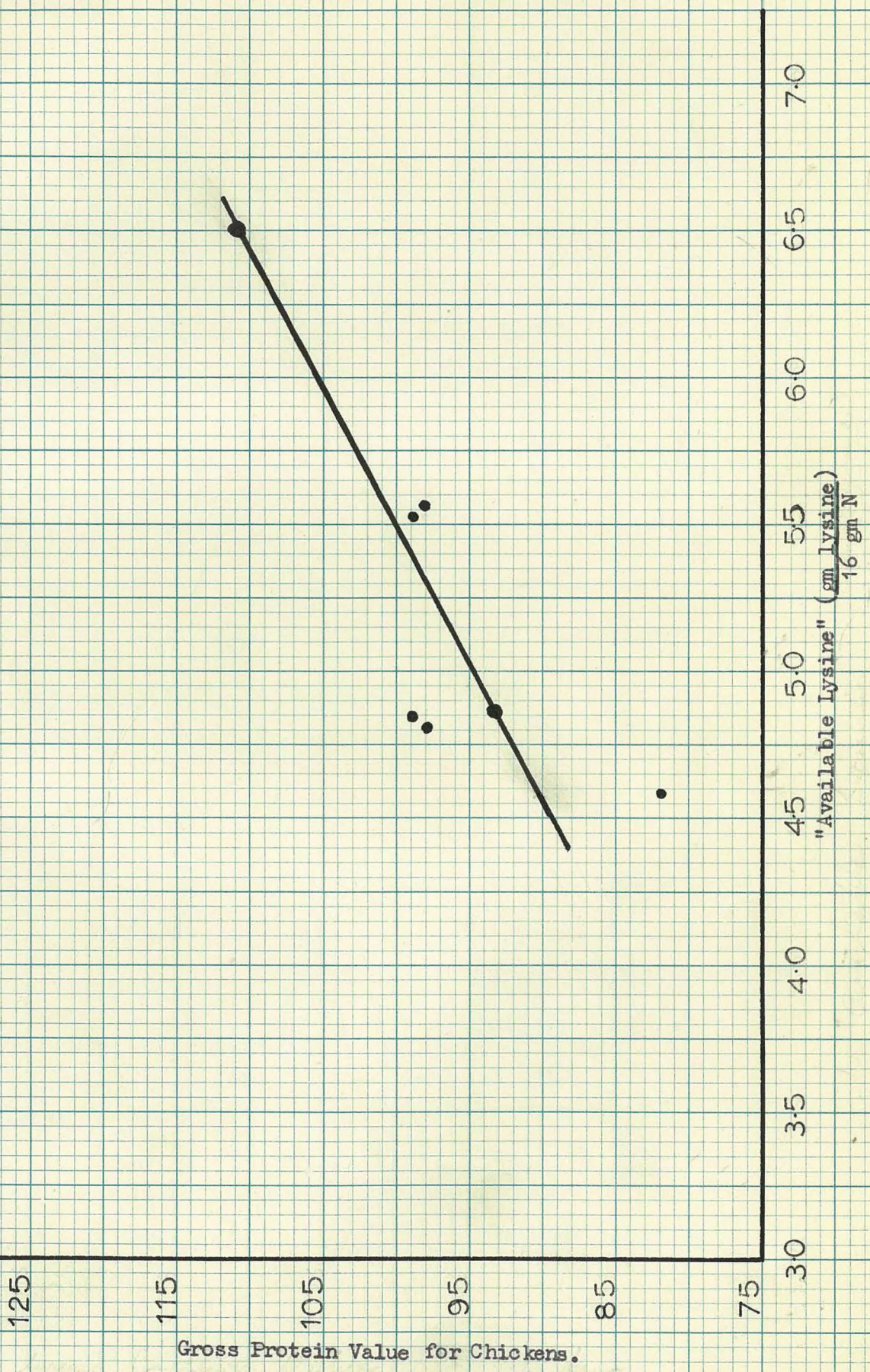
The "available lysine" content of each meatmeal is shown in Table XXXV. Makarewa was shown to contain 6.50 gm lysine per 16 gm N, and Patea 4.57 gm lysine per 16 gm N, while the other meatmeals were found to have intermediate values.

The statistical analysis showed that a significant relationship between the gross protein value and the "available lysine" existed, and a significant correlation coefficient of 0.84 was calculated (see Appendix XIX). An estimation of the gross protein value of the meatmeals may be determined from the regression equation (see Appendix XIX).

$$Y = 42.79 + 10.42 X$$

if the "available lysine" value of the meatmeal is known.

Graph V: The Gross Protein Value and "Available Lysine".



Gross Protein Value for Chickens.

CHAPTER IV

DISCUSSION

In poultry diets the meatmeals which are used as a source of supplementary protein may contribute up to 50% of the total protein and most of calcium, as well as some of the phosphorus of the final diet. Therefore any variations in the protein quality and the composition of the meatmeals would influence the nutritive value of the final diet.

In this study the meatmeals under test were fed with a cereal basal ration so that their nutritive value could be determined when they were used as a source of supplementary protein. The differences recorded in the growth rate of the chickens fed these diets were really a measure of the nutritive value of the experimental diets, but because the cereal basal ration was the same for all diets these recorded differences in growth rate also reflected the nutritive value of the meatmeals studied.

(1) The Factors Affecting the Nutritive Value of Meatmeals.

The results of Experiment I showed that the nutritive value of the meatmeals was influenced by several factors because the statistical analysis of the regression lines divided up the meatmeals studied into two groups. These results are in agreement with other findings (McDonald, 1961). Also significant differences were found between the nutritive values of the Group I meatmeals (see Table XX).

It was shown that the variation in the ash content gave a direct measure of the variation in the levels of calcium and phosphorus, as high

correlations were found to exist between the ash content and the level of calcium ($r = + 0.96$), and the level of phosphorus ($r = + 0.91$) in meatmeals. In the meatmeals studied the ash content was found to vary from 3.22% to 32.30%. As a result, a wide variation in the levels of calcium and phosphorus was found in the experimental diets of Experiment I.

An examination of the diets of the Group I meatmeals (Pareora, Longburn, Belfast, Fairfield, Gear, Wanganui, Gisborne and Patea) and the Group II meatmeals (Makarewa and Waitara) in Experiment I was made to see whether the variation found in the calcium and phosphorus levels was one of the factors influencing the nutritive value of the meatmeals. The examination showed that there was very little variation between the calcium and phosphorus levels within Group II diets, and that these levels of calcium and phosphorus did not fall outside the range of levels found in Group I diets. It was concluded that under the conditions of Experiment I the difference found between the Group I and Group II meatmeals, as well as the difference shown in nutritive value within the Group I meatmeals, was not due to the variation in the calcium and phosphorus levels. This conclusion was confirmed by the results of Experiment II.

These results were not in agreement with the recent work (Smith, et al. 1961) which showed that the growth rate of the chickens was depressed when they were fed a diet in which the level of calcium was increased from 0.83% to 1.35%, while the results of Experiment II showed that no significant differences in the growth rate of the chickens occurred when the level of calcium in a diet containing 0.8% phosphorus was increased from 0.5% to 1.9%. The difference in results could possibly be explained in terms of the length of the experimental period, because in Experiment II the chickens were only fed the diets containing different levels of calcium for two weeks, as compared with six and ten weeks in the Experiment of (Smith, et al. 1961). Therefore, it appeared that the two week experimental period was probably too short to

allow for the effect of the varying levels of calcium to significantly depress the growth rate of the chickens in Experiment I. However, it must be mentioned that under the normal conditions of raising chickens in New Zealand, the results of the recent work would seem to suggest that the ash content of the meatmeals could be an important factor influencing their nutritive value.

In Experiment I the experimental diets were found to have a calcium to phosphorus ratio of approximately 2:1 which has been shown to be satisfactory for chickens (Morrison, 1959) and, therefore, any differences observed in the nutritive value of the meatmeals were not due to an unfavourable calcium phosphorus ratio. The effect of a high calcium phosphorus ratio (4.6:1) on the growth rate of the chickens was demonstrated by the results of Experiment II, which showed that the growth rate was greatly depressed when the calcium level was increased to 1.4% on a diet containing 0.3% phosphorus.

The variation in the protein quality of the meatmeal is another important factor which would affect the nutritive value of the meatmeals. The protein quality is influenced by the variable nature of the raw materials used for the processing of meatmeals and the severity of the heat treatment applied during the processing of these raw materials. It has been shown (Hoagland, et al. 1926A, 1926B, and 1932) that the use of raw materials which are high in connective tissue for the processing of meatmeals would result in the production of meatmeals of low nutritive value, because the connective tissue is made up of the proteins gelatin and elastin which are low in the essential amino acids (Sahyun, 1948). The loss in the nutritive value of protein foodstuffs such as meatmeals, caused by the application of heat during their processing, is a result of the destruction or a reduction

in the availability of the amino acids such as lysine (Patton, 1950). The effect of heat on lysine was demonstrated in Experiment III where it was shown that the "available lysine" was progressively reduced as the severity of the autoclaving treatment was increased. No significant differences in the nutritive value, however, were found between the various samples of autoclaved meat in Experiment III. This was probably due to the fact that the two week experimental period was too short to allow for the significant differences to show, as it has been demonstrated by (Seuk, et al. 1949 and Morgan, et al. 1934) that when samples of autoclaved meat were fed to rats for a period of four to six weeks significant differences in the nutritive value of the samples were found.

The reduction in the "available lysine" during the heating was the result of the free ϵ -amino groups of the lysine which were not involved in peptide linkages reacting with other active groups and so protecting the lysine from the action of the 2,4 - dinitrofluorobenzene. Meats and meatmeals contain very little carbohydrate and, therefore, it appeared that the most important reaction involved in the reduction of the availability of lysine was the non-carbohydrate retention reaction (Almquist, 1957 and Pader, et al. 1948). This reaction involves the free ϵ -amino groups of the lysine and other active groups such as the carboxyl groups of aspartic and glutamic acid (Eldred, et al. 1946). Since some carbohydrates are present in meat the Maillard reaction which involves the free sugars and amino acids would also account for part of the reduction in the "available lysine", because the free ϵ -amino groups of the lysine could have reacted with the free carbohydrates (Lea, 1948 and Hodge, 1953). Although the reduction in the "available lysine" is accelerated by increasing the temperature and cooking intervals (Experiment III), it was shown that the lysine was not reduced to

a level where it became the limiting amino acid in meatmeal (Experiment IV and Kratzer, et al. 1959).

Finally, from recent work (McDonald, et al 1959), a factor was isolated from the mineral fraction of meat and bone meals which was found to be responsible for the reduction of the nutritive value of these meals. The mode of action of this factor was thought to be through oxidative changes in the meals because the addition of an anti-oxidant was shown to improve the nutritive value of these meat and bone meals. It is possible that a factor similar to that mentioned above (McDonald, et al. 1959) could have been responsible for the difference between the Group I and Group II meatmeals, as well as the differences found between the nutritive value of the Group I meatmeals, but further work on this aspect is necessary before anything conclusive can be said for N.Z. meatmeals.

2. The Chemical Evaluation of the Nutritive Value of Meatmeals.

A significant correlation between the gross protein value and the "available lysine" was found to exist for the seven meatmeals studied. This finding agrees with recent work (Carpenter, et al. 1955) which showed that the same relationship existed in other animal products such as fishmeals and whale by-products. The establishment of a correlation between the "available lysine" and the gross protein value could be reasonably expected in animal products because the severe effects of processing do cause a reduction in the nutritional availability of the lysine. Further, the major reason for the high value of proteins made from animal tissues is due to their high content of "available lysine" and, therefore, animal products which are found to be low in "available lysine" will most likely have a low gross protein value.

The above relationship between the "available lysine" and the gross protein show that the "available lysine" technique could be used as a

possible method to evaluate chemically the protein quality of the meatmeals. A chemical method for the evaluation of protein quality of the meatmeals would enable routine determinations to be carried out to check on the protein quality of the meatmeals. The present biological methods involving small animals are not suitable for routine determinations of protein quality because of the time and expense involved. However, before the "available lysine" technique can be considered as a suitable method for the chemical evaluation of the protein quality of meatmeals, further determinations will have to be carried out to substantiate the results of Experiment V and to develop a suitable regression equation.

The findings of Experiment V would suggest that lysine is the limiting amino acid under the conditions of the gross protein value test. This observation may appear to be contradictory to the previous work (Experiment IV and Krazer, et al. 1959) which showed that lysine was not the limiting amino acid in meatmeals. However, the apparent anomaly of results may be explained when it is pointed out that the meatmeals were studied under different conditions. In Experiment IV the meatmeal was the major source of protein studied, where as in the gross protein value diets the meatmeals studied were fed with a cereal basal ration and, therefore, the mutual supplementary action (Almquist, 1957) of the cereal and meatmeal protein must be considered. The effect of the mutual supplementary action between the meatmeal and the cereal proteins could have resulted in the lysine becoming the limiting amino acid in the gross protein value diets because the cereals are deficient in lysine (Almquist, 1957 and McDonald, 1961). Support for this idea was shown by the results of some work (March, et al. 1950) which showed that lysine could be the limiting amino acid in cereal meatmeal diets. However, the possibility that some other amino acid may be limiting and that it occurs in some fixed relationship with the

"available lysine" must also be considered as a possible explanation.

Finally, low levels of protein are employed in the determinations of the gross protein value to emphasize the differences in quality which might not attain statistical significance. The emphasis is useful where uniformity amongst experimental animals is difficult to attain and differences in protein quality may be expected to be small.

CHAPTER V

SUMMARY

1. The meatmeals were shown to be heterogenous products which contained varying amounts of protein, fat, ash, and moisture.
2. Experimental groups of chickens were used to determine the nutritive values of the meatmeals. These meatmeals were fed as a source of supplementary protein with a cereal basal ration.
3. Significant differences were found between the nutritive values determined for the various samples of meatmeals.
4. The differences shown in the nutritive values of the meatmeals could not be related to a single nutrient.
5. During a two week experimental period it was shown that in diets containing 0.3% phosphorus an increase in the calcium level to 1.4% resulted in a significant depression in the growth rate of the chickens. Under the same experimental conditions it was also shown that an increase in the calcium level from 0.5% to 1.9% in diets containing 0.8% phosphorus did not cause a significant depression in the growth rate of the chickens.

6. The amino acid lysine was shown not to be the limiting amino acid in meatmeals.

7. The "available lysine" in meat was shown to be progressively reduced as the severity of the autoclaving treatment was increased.

8. A significant correlation was found to exist between the "available lysine" and the gross protein value in meatmeals.

9. If further work confirms the findings of this study then the 2,4 -dinitrofluorobenzene technique for the estimation of the "available lysine" could be used as a suitable method for the chemical evaluation of the protein quality in New Zealand meatmeals.

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APPENDIX XVIII

The Gross Protein Value Data.

APPENDIX XIX

The Correlation Between the Gross Protein Value and "Available Lysine".

APPENDIX XX

The Correlation Between the Ash and Calcium, and the Ash and Phosphorus in Meatmeals.

APPENDICES

APPENDIX I

Experiment I: The Conditions under which the Meatmeals Studied were Processed.

- (a) Gear Dry rendering. The process used a pressure of 80 lbs./sq.in. (324°F) in the outer jackets, during which the material was vented for an hour, after which the pressure inside allowed to build up to 30 lbs./sq.in. (274°F) and held for one hour. The pressure inside was then relieved while the pressure in the outer jacket was maintained until the material was dry.
- (b) Waitara Dry rendering. The outer jacket pressure was maintained at 80 lbs./sq.in (324°F), and the internal pressure was allowed to rise to 40 lbs./sq.in. (290°F) and held for approximately an hour. The internal pressure was then released and the material dried.
- (c) Waitaki Dry rendering. The material was cooked for 2 hrs. at 312°F.
- (d) Makarewa Dry rendering. The material was vented for 1½-2 hrs. before the internal pressure was allowed to rise to 35 lbs./sq.in. (280°F).

- (e) Patea Dry rendering. The material was cooked for 3 hrs. at 40 lbs./sq.in. (290°F).
- (f) Wanganui Wet rendering. The material was cooked at a temperature of 367°F - 370°F.
- (g) Gisborne Dry rendering. During processing a pressure of 80 lbs./sq.in. (324°F) was used in the outer jacket. After venting for an hour the internal jacket pressure was raised to 30 lbs./sq.in. (274°F) and maintained for an hour before the pressure was reduced. The pressure in the outer jacket was maintained until the material was dry.
- (h) Pareora, Belfast and Fairfield. Dry rendering. The total "cooking" varies from about 4 hrs. to 4 $\frac{3}{4}$ hrs. according to the nature of the charge. The temperature of the material was raised from cold to 212°F and was held there until most of the moisture had been evaporated. This took approximately 2 hrs. from the start. The machine was then closed down and its internal pressure was raised to 35 lbs./sq.in. (280°F), held for $\frac{1}{2}$ hr. then lowered.

All the data worked in Experiments I, III and IV was worked in log terms. In Graphs I, II and III the protein intake values were plotted in log terms to give a straight line graph, because the growth data when plotted in normal terms would follow an exponential growth curve. Also, to make the data used for the calculations easier to work with, the characteristics of the log protein intake values were increased by one because some of the characteristics of these values were less than nought.

APPENDIX II

Trial I - Growth Rate and Food Intake Data.

Experiment I:

Longburn

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.136	1.0553	1.854	1.2681	2.969	1.4726
Weekly Weight Gain (gms) per chicken.	16.20	1.2095	17.90	1.2529	36.80	1.5658
	11.15	1.0472	16.90	1.2279	44.30	1.6464
	14.65	1.1659	20.60	1.3139	38.60	1.5866
	17.45	1.2417	20.10	1.3032	34.05	1.5321
	13.15	1.1189	24.10	1.3820	47.05	1.6726
	18.40	1.2648	29.70	1.4728	34.95	1.5434
	10.25	1.0107	17.35	1.2392	31.70	1.5011
	14.00	1.1461	22.10	1.3444	40.60	1.6085
	14.30	1.1553	24.00	1.3820	27.00	1.4314
Total Weight Gain	129.55	10.3601	192.75	11.9183	335.05	14.0879

$$\begin{aligned} \sum X &= 34.1640 & \sum X^2 &= 44.01258 & \sum XY &= 46.79245 & \sum Y^2 &= 49.91242 \\ \sum Y &= 36.3663 & C &= 43.22884 & &= 46.01549 & &= 48.98176 \\ & & \sum x^2 &= 0.78374 & \sum xy &= 0.77696 & \sum y^2 &= 0.93066 \end{aligned}$$

$$b = 0.99$$

Gear

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.439	1.1580	2.734	1.4368	4.247	1.6281
Weekly Weight Gain (gms) per chicken.	18.80	1.2742	23.00	1.3617	52.45	1.7197
	12.45	1.0952	26.50	1.4232	41.15	1.6143
	13.95	1.1446	21.75	1.3375	39.20	1.5933
	15.00	1.1761	36.45	1.5617	30.80	1.4886
	10.70	1.0294	24.20	1.3838	54.70	1.7380
	12.45	1.0952	33.45	1.5244	60.60	1.7825
	13.55	1.1319	33.20	1.5211	36.85	1.5664
	8.50	0.9294	30.45	1.4836	65.20	1.8142
	16.30	1.2122	33.45	1.5244	56.05	1.7486
	Total Weight Gain	121.70	10.0882	262.45	13.1214	437.00

$$\sum X = 38.0061$$

$$\sum X^2 = 54.50461$$

$$\sum XY = 55.06326$$

$$\sum Y^2 = 55.89310$$

$$\sum Y = 38.2752$$

$$C = 53.49864$$

$$= 53.87744$$

$$= 54.25892$$

$$\sum x^2 = 1.00597$$

$$\sum xy = 1.18582$$

$$\sum y^2 = 1.63418$$

$$b = 1.18$$

Waitaki

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.397	1.1452	2.162	1.3349	2.687	1.4292
Weekly Weight Gain (gms) per chicken	16.40	1.2148	23.15	1.3645	30.50	1.4843
	17.20	1.2355	32.10	1.5065	32.60	1.5132
	14.45	1.1599	22.05	1.3434	23.75	1.3756
	13.05	1.1155	26.00	1.4150	17.85	1.2516
	14.75	1.1688	26.35	1.4208	37.05	1.5688
	14.15	1.1507	31.15	1.4935	25.00	1.3979
	17.00	1.2304	29.95	1.4764	35.95	1.5557
	13.55	1.1319	33.40	1.5237	38.00	1.5798
	11.50	1.0607	26.35	1.4208	43.45	1.6380
Total Weight Gain	132.05	10.4682	250.50	12.9646	284.15	13.3649

$$\sum X = 35.1837$$

$$\sum X^2 = 46.22449$$

$$\sum XY = 48.39574$$

$$\sum Y^2 = 50.87591$$

$$\sum Y = 36.7977$$

$$C = 45.84787$$

$$= 47.95108$$

$$= 50.15078$$

$$\sum x^2 = 0.37662$$

$$\sum xy = 0.44466$$

$$\sum y^2 = 0.72513$$

$$b = 1.18$$

Pareora

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.429	1.1550	2.087	1.3196	3.988	1.6008
Weekly Weight Gain (gms) per chicken.	8.65	0.9370	39.10	1.5922	53.70	1.7300
	13.50	1.1303	19.25	1.2844	64.40	1.8089
	13.20	1.1206	22.65	1.3551	52.10	1.7168
	12.30	1.0899	32.00	1.5051	51.85	1.7147
	16.25	1.2108	10.90	1.0374	43.95	1.6430
	12.20	1.0864	43.20	1.6355	64.30	1.8082
	12.45	1.0952	12.55	1.0987	61.35	1.7879
	22.50	1.3522	39.20	1.5933	44.05	1.6440
	14.90	1.1732	16.65	1.2214	59.80	1.7767
Total Weight Gain	125.95	10.1956	235.50	12.3231	495.50	15.6302

$$\sum X = 36.6786$$

$$\sum = 38.1489$$

$$\sum X^2 = 50.74137$$

$$C = 49.82665$$

$$\sum x^2 = 0.91472$$

$$\sum XY = 53.05830$$

$$= 51.82400$$

$$\sum xy = 1.23430$$

$$\sum Y^2 = 56.10245$$

$$= 53.90142$$

$$\sum y^2 = 2.20103$$

$$b = 1.35$$

Belfast

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.251	1.0972	2.068	1.3156	3.583	1.5543
Weekly Weight Gain (gms) per chicken.	15.55	1.1917	33.35	1.5231	53.90	1.7316
	10.70	1.0294	28.60	1.4564	52.05	1.7164
	12.65	1.1021	29.15	1.4646	56.35	1.7509
	13.95	1.1446	31.80	1.5024	62.65	1.7969
	11.30	1.0531	34.65	1.5397	43.20	1.6355
	13.30	1.1239	25.70	1.4099	40.85	1.6112
	18.05	1.2565	25.40	1.4048	51.70	1.7135
	7.80	0.8921	23.15	1.3645	46.60	1.6684
	14.05	1.1476	21.20	1.3263	58.55	1.7676
Total Weight Gain	117.35	9.9410	253.00	12.9917	465.85	15.3920

$$\sum X = 35.7039$$

$$\sum X^2 = 48.15449$$

$$\sum XY = 51.92293$$

$$\sum Y^2 = 56.21826$$

$$\sum X = 38.3247$$

$$C = 47.21364$$

$$= 50.67930$$

$$= 54.39935$$

$$\sum x^2 = 0.94085$$

$$\sum xy = 1.24363$$

$$\sum y^2 = 1.81891$$

$$b = 1.32$$

Fairfield

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.237	1.0923	2.119	1.3261	3.414	1.5333
Weekly Weight Gain (gms) per chicken.	19.00	1.2788	17.10	1.2330	59.35	1.7735
	9.35	0.9708	19.55	1.2911	54.05	1.7328
	12.30	1.0899	41.10	1.6138	52.55	1.7206
	21.35	1.3294	37.60	1.5752	40.75	1.6101
	13.80	1.1399	32.00	1.5051	56.05	1.7486
	15.10	1.1790	22.45	1.3512	49.95	1.6985
	15.75	1.1973	41.50	1.6180	65.60	1.8169
	11.60	1.0645	30.75	1.4878	53.45	1.7279
	14.55	1.1629	34.65	1.5397	64.95	1.8125
Total Weight Gain	132.80	10.4125	276.70	13.2149	496.70	15.6414

$$\sum X = 35.5653$$

$$\sum Y = 39.2688$$

$$\sum X^2 = 47.72402$$

$$C = 46.84779$$

$$\sum x^2 = 0.87623$$

$$\sum XY = 52.88081$$

$$= 51.72617$$

$$\sum = 1.15464$$

$$\sum Y^2 = 58.92266$$

$$= 57.11254$$

$$\sum y^2 = 1.81012$$

$$b = 1.32$$

Trial II - Growth Rate and Food Intake Data

Longburn

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.228	1.0892	1.766	1.2470	3.092	1.4903
Weekly Weight Gain (gms) per chicken.	22.15	1.3454	26.95	1.4306	37.10	1.5682
	11.75	1.0700	20.25	1.3065	36.25	1.5593
	13.35	1.1255	29.30	1.4669	34.60	1.5391
	13.00	1.1139	33.70	1.5276	26.55	1.4240
	12.45	1.0952	28.05	1.4480	44.50	1.6484
	13.45	1.1287	19.70	1.2945	25.25	1.4023
	13.60	1.1335	16.50	1.2175	29.50	1.4698
	9.40	0.9731	34.25	1.5346	39.15	1.5928
	21.70	1.3365	23.80	1.3766	31.70	1.5011
	16.40	1.2148	19.45	1.2889	39.60	1.5977
Total Weight Gain	147.25	11.5366	251.95	13.8917	344.20	15.3027

$$\Sigma X = 38.2650$$

$$\Sigma Y = 40.7310$$

$$\Sigma x^2 = 49.62359$$

$$\Sigma y^2 = 48.80700$$

$$\Sigma x^2 = 0.81659$$

$$\Sigma XY = 52.69423$$

$$= 51.95239$$

$$\Sigma xy = 0.74184$$

$$\Sigma Y^2 = 56.30882$$

$$= 55.30047$$

$$\Sigma y^2 = 1.00835$$

$$b = 0.91$$

Wanganui

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.192	1.0762	2.137	1.3298	2.816	1.4496
Weekly Weight Gain (gms) per chicken.	18.20	1.2601	30.45	1.4836	38.95	1.5905
	10.30	1.0128	18.70	1.2718	39.40	1.5955
	16.95	1.2292	25.50	1.4065	51.30	1.7101
	15.95	1.2028	17.00	1.2304	42.65	1.6299
	16.95	1.2292	26.85	1.4289	52.35	1.7189
	11.60	1.0645	35.65	1.5520	51.55	1.7122
	12.60	1.1004	20.60	1.3139	44.10	1.6444
	14.60	1.1644	35.85	1.5545	33.10	1.5198
	12.70	1.1038	19.80	1.2967	43.45	1.6380
	15.65	1.1945	20.40	1.3096	30.00	1.4771
Total Weight Gain	145.50	11.5617	250.80	13.8479	426.85	16.2364

$$\Sigma X = 38.5560$$

$$\Sigma Y = 41.6460$$

$$\Sigma X^2 = 50.27914$$

$$C = 49.55217$$

$$\Sigma x^2 = 0.72697$$

$$\Sigma XI = 54.39392$$

$$= 53.52343$$

$$\Sigma xy = 0.87049$$

$$\Sigma Y^2 = 59.14898$$

$$= 57.81297$$

$$\Sigma y^2 = 1.33601$$

$$b = 1.20$$

Patea

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.124	1.0508	1.559	1.1928	2.316	1.3647
Weekly Weight Gain (gms) per chicken.	10.10	1.0043	11.00	1.0414	33.60	1.5263
	12.55	1.0987	22.05	1.3434	19.20	1.2833
	10.30	1.0128	32.60	1.5132	33.45	1.5244
	11.15	1.0473	16.20	1.2095	39.95	1.6016
	9.00	0.9542	13.80	1.1399	11.50	1.0607
	10.05	1.0021	22.55	1.3532	37.20	1.5705
	15.80	1.1987	35.05	1.5447	45.20	1.6551
	13.80	1.1399	28.50	1.4548	18.20	1.2601
	14.50	1.1614	27.60	1.4409	33.55	1.5257
	16.85	1.2266	17.20	1.2355	9.20	0.9638
Total Weight Gain	124.10	10.8460	226.55	13.2765	281.05	13.9715

$$\sum X = 36.0830$$

$$\sum Y = 38.0940$$

$$\sum X^2 = 43.89359$$

$$C = 43.39942$$

$$\sum x^2 = 0.49417$$

$$\sum XY = 46.30009$$

$$= 45.81819$$

$$\sum xy = 0.48190$$

$$\sum Y^2 = 49.76114$$

$$= 48.37176$$

$$\sum y^2 = 1.38938$$

$$b = 0.98$$

Gisborne

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.263	1.1014	2.449	1.3890	3.430	1.5353
Weekly Weight Gain (gms) per chicken.	15.90	1.2014	28.85	1.4602	45.30	1.6561
	14.80	1.1703	47.90	1.6803	55.05	1.7408
	16.05	1.2054	23.65	1.3738	64.85	1.8119
	19.60	1.2923	31.80	1.5024	45.10	1.6542
	16.65	1.2214	22.05	1.3434	50.65	1.7046
	9.10	0.9590	38.15	1.5815	43.55	1.6390
	14.50	1.1614	22.25	1.3474	66.95	1.8257
	9.25	0.9661	24.45	1.3883	26.55	1.4240
	8.05	0.9058	28.55	1.4556	51.50	1.7118
	16.35	1.2135	24.50	1.3892	63.75	1.8044
Total Weight Gain	140.25	11.2966	292.15	14.5221	513.25	16.9725

$$\sum X = 40.2570$$

$$\sum Y = 42.7912$$

$$\sum X^2 = 54.99549$$

$$C = 54.02086$$

$$\sum x^2 = 0.97463$$

$$\sum XY = 58.67115$$

$$= 57.42151$$

$$\sum xy = 1.24964$$

$$\sum Y^2 = 63.05172$$

$$= 61.03622$$

$$\sum y^2 = 2.01550$$

$$b = 1.28$$

Makarewa

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.360	1.1335	2.338	1.3689	3.573	1.5531
Weekly Weight Gain (gms) per chicken.	12.00	1.0792	30.60	1.4857	50.30	1.7016
	12.55	1.0987	23.00	1.3617	62.80	1.7980
	13.30	1.1239	22.70	1.3560	62.20	1.7938
	22.60	1.3541	40.80	1.6107	53.10	1.7251
	24.10	1.3820	40.85	1.6112	54.35	1.7352
	12.50	1.0969	29.20	1.4654	43.80	1.6415
	10.00	1.0000	27.55	1.4401	57.55	1.7601
	14.05	1.1476	37.30	1.5717	39.05	1.5917
	10.95	1.0395	22.45	1.3512	78.35	1.8941
	11.25	1.0511	24.50	1.3892	71.70	1.8555
Total Weight Gain	143.30	11.3730	298.95	14.6429	573.20	17.4966

$$\Sigma X = 40.5550$$

$$\Sigma Y = 43.5125$$

$$\Sigma x^2 = 55.70829$$

$$C = 54.82360$$

$$\Sigma x^2 = 0.88469$$

$$\Sigma XY = 60.10993$$

$$= 58.82164$$

$$\Sigma xy = 1.28829$$

$$\Sigma Y^2 = 65.31108$$

$$= 63.11125$$

$$\Sigma y^2 = 2.19983$$

$$b = 1.46$$

Waitara

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.478	1.1697	2.078	1.3177	2.925	1.4661
Weekly Weight Gain (gms) per chicken.	16.60	1.2201	13.45	1.1287	50.95	1.7071
	10.70	1.0294	36.25	1.5593	41.85	1.6217
	12.65	1.1022	30.50	1.4843	35.85	1.5545
	17.40	1.2405	19.70	1.2945	46.20	1.6646
	6.55	0.8162	16.40	1.2148	45.45	1.6575
	11.55	1.0625	25.50	1.4065	31.35	1.4962
	13.00	1.1139	23.35	1.3683	42.45	1.6279
	14.05	1.1476	29.75	1.4735	30.00	1.4771
	14.80	1.1703	29.35	1.4676	41.70	1.6201
	10.75	1.0315	17.20	1.2355	62.25	1.7941
Total Weight Gain	128.05	10.9342	241.45	13.6330	428.05	16.2208

$$\sum X = 39.5350$$

$$\sum X^2 = 52.53980$$

$$\sum XY = 54.53525 \quad \sum Y^2 = 57.24322$$

$$\sum Y = 40.7880$$

$$C = 52.10054$$

$$= 53.75178 \quad = 55.45536$$

$$\sum x^2 = 0.43926$$

$$\sum xy = 0.78347$$

$$\sum y^2 = 1.78786$$

$$b = 1.78$$

APPENDIX III

Experiment I:

Data Plotted for Graphs I and II.

Protein Level	Average Weekly Weight Gain in gms. per chicken.			Daily Protein Intake per chicken in gms.			Log Daily Protein Intake per chicken in gms.		
	11%	15%	20%	11%	15%	20%	11%	15%	20%
	<u>TRIAL I</u>								
Longburn	14.39	21.42	37.23	1.136	1.854	2.969	1.0553	1.2681	1.4726
Gear	13.52	29.16	48.55	1.439	2.734	4.247	1.1580	1.4368	1.6281
Waitaki	14.67	27.83	31.57	1.397	2.162	2.687	1.1452	1.3349	1.4292
Pareora	13.99	26.17	55.06	1.429	2.087	3.988	1.1550	1.3196	1.6008
Belfast	13.04	28.11	51.76	1.251	2.068	3.583	1.0972	1.3156	1.5543
Fairfield	14.76	30.74	55.19	1.237	2.119	3.414	1.0923	1.3261	1.5333
	<u>TRIAL II</u>								
Longburn	14.73	25.20	34.42	1.228	1.766	3.092	1.0892	1.2470	1.4903
Wanganui	14.55	25.08	42.69	1.192	2.137	2.816	1.0762	1.3298	1.4496
Gisborne	14.03	29.22	51.33	1.263	2.449	3.430	1.1014	1.3890	1.5353
Waitara	12.81	24.15	42.81	1.478	2.078	2.925	1.1697	1.3177	1.4661
Patea	12.41	22.70	28.11	1.124	1.559	2.316	1.0508	1.1928	1.3647
Makarewa	14.33	29.89	57.32	1.360	2.338	3.573	1.1335	1.3689	1.5531

APPENDIX IV

Experiment I:

* Covariance Analysis

Longburn Trial I			
$\sum X = 34.1640$	$\sum X^2 = 44.01258$	$\sum XY = 46.79245$	$\sum Y^2 = 49.91242$
$\sum Y = 36.3663$	$C = 43.22884$	$C = 46.01549$	$C = 48.98176$
	$\sum x^2 = 0.78374$	$\sum xy = 0.77696$	$\sum y^2 = 0.93066$
Longburn Trial II			
$\sum X = 38.2650$	$\sum X^2 = 49.62359$	$\sum XY = 52.69423$	$\sum Y^2 = 56.30882$
$\sum Y = 40.7310$	$C = 48.80700$	$C = 51.95239$	$C = 55.30047$
	$\sum x^2 = 0.81659$	$\sum xy = 0.74184$	$\sum y^2 = 1.00835$
Total N = 57			
$\sum X = 72.4290$	$\sum X^2 = 93.63617$	$\sum XY = 99.48668$	$\sum Y^2 = 106.22124$
$\sum Y = 77.0973$	$C = 92.03438$	$C = 97.96632$	$C = 104.28059$
	$\sum x^2 = 1.60179$	$\sum xy = 1.52036$	$\sum y^2 = 1.94065$

* (Snedecor, 5th edition)

Experiment I:

* Covariance Analysis

Makarna			
$\sum X = 40.5550$	$\sum X^2 = 55.70829$	$\sum XY = 60.10993$	$\sum Y^2 = 65.31108$
$\sum Y = 43.5125$	$C = 54.82360$	$C = 58.82164$	$C = 63.11125$
	$\sum x^2 = 0.88469$	$\sum xy = 1.28829$	$\sum y^2 = 2.19983$
Waltari			
$\sum X = 39.5350$	$\sum X^2 = 52.53980$	$\sum XY = 54.53525$	$\sum Y^2 = 57.24322$
$\sum Y = 40.7880$	$C = 52.10054$	$C = 53.75178$	$C = 55.45536$
	$\sum x^2 = 0.43926$	$\sum xy = 0.78347$	$\sum y^2 = 1.78786$
Total N =60			
$\sum X = 80.0900$	$\sum X^2 = 108.24809$	$\sum XY = 114.64518$	$\sum Y^2 = 122.55430$
$\sum Y = 84.3005$	$C = 106.90680$	$C = 112.52711$	$C = 118.44290$
	$\sum x^2 = 1.34129$	$\sum xy = 2.11807$	$\sum y^2 = 4.11140$

APPENDIX V

Experiment I: The Fiducial Limits of the Regression Coefficients of the Meatmeals, taking Longburn II as an example:

The sample standard deviation of the Regression Coefficient = $S_b = \frac{S_{y.x}}{\sqrt{\sum x^2}}$

$$\sum d y.x^2 = 0.3344 \quad \text{d.f.} = 28 \quad \sum x^2 = 49.623$$

$$S_{y.x^2} = \frac{\sum d y.x^2}{N-2}$$

$$S_{y.x} = \sqrt{\frac{\sum d y.x^2}{N-2}}$$

$$S_b = \sqrt{\frac{\frac{\sum d y.x^2}{N-2}}{\sum x^2}}$$

$$S_b = \sqrt{\frac{\frac{0.3344}{28}}{49.623}} = .0491$$

• The fiducial limits of the regression coefficient are equal to:

$$b - t_{.05} S_b \leq \beta \leq b + t_{.05} S_b$$

• 95% Fiducial Limits = $\pm S_b \times t_{.05}$ for 28 d.f. ($t_{.05}$ for 28 d.f. = 2.048)
 = $.0491 \times 2.048 = 0.100$

The fiducial limits for the regression coefficient of Longburn II = ± 0.10

APPENDIX VI

Experiment I: The Calculation of the Relative Potency of the Meatmeals.

The formula used to calculate the Relative Potency, where M is the relative potency in log terms, is given below:

$$M = \bar{X}_S - \bar{X}_T - \frac{\bar{Y}_S - \bar{Y}_T}{b}$$

Longburn is the standard.

For example, comparing Longburn (S) with Gear (T)

$$M = 1.2653 - 1.4076 - \frac{(1.3469 - 1.4176)}{1.2331}$$

$$M = -0.1423 - \frac{(-0.0707)}{1.2331}$$

$$M = -0.1423 + 0.0573$$

$$M = -0.0850$$

R is relative potency in ordinary terms.

$$R = \frac{1}{\text{antilog } M} \quad (\text{when } M \text{ is negative})$$

$$\text{or } R = \text{antilog } M \quad (\text{when } M \text{ is positive})$$

$$R = \frac{1}{1.216} = 0.822 \quad (82.2)$$

The relative potency given in Table XIX is calculated by multiplying R by 100.

APPENDIX VII

Experiment I: The Calculation of the Fiducial Limits of the Relative Potency of the Meatmeals

The fiducial limits were calculated from the following formula where the Variance of M equals:

$$\sigma^2_M = \frac{s^2}{b^2} \left[\frac{1}{N_S} + \frac{1}{N_T} + \frac{(M - \bar{x}_S + \bar{x}_T)^2}{\sum x^2} \right]$$

because all the comparisons made conformed to the g - criterion.

$$g = \frac{s^2 t^2}{b^2 \sum x^2}$$

The fiducial limits can only be calculated from the above formula if the value of g is less than 0.1

The Values of g for the Comparisons Made.	
<u>TRIAL I</u>	
Longburn v Gear	0.016
Longburn v Waitaki	0.025
Longburn v Pareora	0.017
Longburn v Belfast	0.017
Longburn v Fairfield	0.017
<u>TRIAL II</u>	
Longburn v Wanganui	0.036
Longburn v Gisborne	0.031
Longburn v Patea	0.042

* Burn, et al.

The calculation of the fiducial limits of the relative potency of Gear.

$$s^2 = 0.0108 \quad b^2 = 1.5205 \quad N_G = 27 \quad N_T = 27$$

$$M = -0.0850 \quad \bar{x}_G = 1.2653 \quad \bar{x}_T = 1.4076$$

$$\sum x^2 = 1.7891$$

$$b^2 M = \frac{0.0108}{1.5205} \quad 0.0741 + \frac{(-0.0850 - 1.2653 + 1.4076)^2}{1.7891}$$

$$b^2 M = 0.0071 \quad (0.0759)$$

$$b M = \sqrt{0.000539}$$

$$b M = 0.02321$$

Fiducial Limits

$$= \pm 0.02321 \times 2.006 = 0.046 \quad (t_{.05} \text{ for } 52 \text{ d.f.} = 2.006)$$

$$\therefore 95\% \text{ Limits of } M = -0.0384 \quad \text{and} \quad -0.1316$$

$$\therefore 95\% \text{ Limits of } R = \frac{1}{1.092} \quad \text{and} \quad \frac{1}{1.354}$$

$$= 0.916 \quad \text{and} \quad 0.738$$

Therefore, the relative potency of Gear and its fiducial limits is:

91.6

82.2

73.8

APPENDIX VIII

Experiment I: A Comparison Between the Relative Potencies of the Meatmeals.

The formula used to compare the relative potencies of the meatmeals for significant differences was:

$$M_1 - M_2 > t_{.05} (N - 2) \text{ d.f.} \sqrt{\sigma^2 M_1 + \sigma^2 M_2}$$

where M_1 and M_2 are the relative potencies in log terms, while $\sigma^2 M_1$ and $\sigma^2 M_2$ are the variances of M_1 and M_2 . If $M_1 - M_2$ is greater than $t_{.05} (N - 2) \text{ d.f.} \sqrt{\sigma^2 M_1 + \sigma^2 M_2}$, then the relative potencies of the two meatmeals are significantly different.

For example, when Fairfield was compared to Gear:

<u>Fairfield</u>	<u>Gear</u>
$M_1 = + 0.0353$	$M_2 = -0.0850$
$\sigma M_1 = 0.000559$	$\sigma M_2 = 0.000539$
$M_1 - M_2 = 0.1103$	

while $t_{.05} (N - 2) \sqrt{\sigma^2 M_1 + \sigma^2 M_2} = 2.006 \times .03316 = 0.0665$

∴ the relative potency of Fairfield is significantly greater than Gear.

APPENDIX IX

Experiment II:

Part I - The Growth Rate of the Chickens Measured
on the Calcium Levels of 0.5%, 0.9% and 1.4%
and the Phosphorus Levels of 0.3% and 0.8%.

Treatment		The Average Gain in Weight in 14 days in gms.	
<u>BLOCK A</u>	0.5% Ca 0.8% P	125.08	
	0.9% Ca 0.8% P	127.28	
	1.4% Ca 0.8% P	120.45	
	0.5% Ca 0.3% P	135.40	
	0.9% Ca 0.3% P	113.79	
	1.4% Ca 0.3% P	80.26	
	<u>BLOCK B</u>	0.5% Ca 0.8% P	130.30
		0.9% Ca 0.8% P	126.31
		1.4% Ca 0.8% P	132.02
0.5% Ca 0.3% P		115.23	
0.9% Ca 0.3% P		105.40	
1.4% Ca 0.3% P		78.41	

APPENDIX X

Experiment II:

Part II - The Growth Rate of the Chickens Measured on the Calcium Levels of 0.5%, 0.9%, 1.4% and 1.9%, and a Phosphorus Level of 0.8%.

Treatment			The Average Gain in Weight in 14 days in gms.
<u>BLOCK A</u>	0.5% Ca	0.8% P	125.08
	0.9% Ca	0.8% P	127.28
	1.4% Ca	0.8% P	120.45
	1.9% Ca	0.8% P	125.62
<u>BLOCK B</u>	0.5% Ca	0.8% P	130.30
	0.9% Ca	0.8% P	126.31
	1.4% Ca	0.8% P	132.02
	1.9% Ca	0.8% P	120.90

APPENDIX XI.

Experiment II:

The Total Food Consumed in 14 days
per chicken (gms).

Treatment	Block A	Block B
0.5% Ca 0.8% P	268.9	253.7
0.9% Ca 0.8% P	237.0	250.3
1.4% Ca 0.8% P	273.6	257.5
1.9% Ca 0.8% P	255.1	243.3
0.5% Ca 0.3% P	239.1	274.1
0.9% Ca 0.3% P	239.5	249.8
1.4% Ca 0.3% P	218.8	210.8

APPENDIX XII

The Growth Rate and Feed Intake Data of Experiment III.

Experiment III:

Freeze Dried Meat.

Protein Level	11%		15%		20%	
	(log)		(log)		(log)	
Protein Intake (gms) per chicken.	1.396	1.1449	1.954	1.2909	3.210	1.5065
Weekly Weight Gain (gms) per chicken.	17.05	1.2316	21.00	1.3222	61.90	1.7917
	12.80	1.1072	54.00	1.7324	49.70	1.6964
	8.90	0.9494	47.90	1.6803	64.25	1.8078
	10.30	1.0128	38.30	1.5832	57.45	1.7593
	12.15	1.0846	29.80	1.4742	54.80	1.7388
	29.80	1.4742	36.10	1.5575	57.00	1.7559
	15.45	1.1889	41.70	1.6201	32.65	1.5139
	11.65	1.0663	42.75	1.6309	67.55	1.8296
	19.30	1.2856	26.45	1.4224	37.05	1.5688
	-	-	30.80	1.4886	-	-
Total Weight Gain	137.40	10.4006	368.80	15.5118	482.35	15.4622

$$\sum X = 36.7716$$

$$\sum Y = 41.3746$$

$$\sum X^2 = 48.88720$$

$$C = 48.29110$$

$$\sum x^2 = 0.59610$$

$$\sum XY = 55.22563$$

$$= 54.33608$$

$$\sum xy = 0.88955$$

$$\sum Y^2 = 63.08317$$

$$= 61.13776$$

$$\sum y^2 = 1.94541$$

$$b = 1.49$$

5 minutes at 24.0° F.

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	0.998	0.9954	2.221	1.3466	2.347	1.3705
Weekly Weight Gain (gms) per chicken.	19.60	1.2923	39.50	1.5966	49.50	1.6946
	13.75	1.1383	54.15	1.7336	43.55	1.6390
	24.35	1.3865	25.30	1.4031	27.60	1.4409
	27.25	1.4354	18.85	1.2754	50.45	1.7028
	9.95	0.9978	29.25	1.4661	42.15	1.6248
	16.35	1.2135	23.60	1.3729	17.15	1.2342
	9.85	0.9934	48.15	1.6826	53.05	1.7247
	25.45	1.4057	19.25	1.2844	44.25	1.6459
	11.15	1.0462	48.30	1.6839	46.95	1.6717
	-	-	56.55	1.7524	55.10	1.7412
Total Weight Gain	157.70	10.9091	362.90	15.2510	429.75	16.1198

$$\sum X = 36.1296$$

$$\sum X^2 = 45.83340$$

$$\sum XY = 53.48809$$

$$\sum Y^2 = 63.25234$$

$$\sum Y = 42.2799$$

$$C = 45.01200$$

$$= 52.67434$$

$$= 61.64103$$

$$\sum x^2 = 0.82140$$

$$\sum xy = 0.81375$$

$$\sum y^2 = 1.61131$$

$$b = 0.99$$

25 minutes at 240°F.

Protein Level	11%		15%		20%	
	(log)		(log)		(log)	
Protein Intake (gms) per chicken.	1.007	1.0029	1.853	1.2679	2.772	1.4428
Weekly Weight Gain (gms) per chicken.	28.40	1.4533	21.85	1.3395	54.55	1.7368
	21.85	1.3395	40.45	1.6069	62.95	1.7990
	8.60	0.9345	37.00	1.5682	34.60	1.5391
	20.25	1.3065	49.95	1.6985	47.80	1.6794
	8.40	0.9243	46.00	1.6628	45.90	1.6618
	8.05	0.9058	28.15	1.4492	56.15	1.7494
	13.54	1.1315	36.35	1.5605	37.80	1.5775
	20.75	1.3171	14.85	1.1718	32.60	1.5132
	8.20	0.9138	20.75	1.3171	51.65	1.7130
	10.45	1.0191	-	-	-	-
Total Weight Gain	148.49	11.2454	295.35	13.3745	424.00	14.9692

$$\sum X = 34.4253$$

$$\sum X^2 = 43.26124$$

$$\sum XY = 49.83310$$

$$\sum Y^2 = 58.15539$$

$$\sum Y = 39.5891$$

$$C = 42.32504$$

$$= 48.67380$$

$$= 55.97488$$

$$\sum x^2 = 0.93620$$

$$\sum xy = 1.15930$$

$$\sum y^2 = 2.18051$$

$$b = 1.23$$

2 hrs. at 240°F.

Protein Level	11%		15%		20%	
	(log)		(log)		(log)	
Protein Intake (gms) per chicken.	1.212	1.0835	2.204	1.3432	2.882	1.4597
Weekly Weight Gain (gms) per chicken.	10.80	1.0334	18.80	1.2742	40.80	1.6107
	9.05	0.9566	19.05	1.2799	58.30	1.7657
	13.70	1.1367	42.90	1.6325	53.55	1.7288
	27.20	1.4346	22.40	1.3502	57.75	1.7616
	6.85	0.8357	32.45	1.5112	45.25	1.6556
	19.85	1.2978	44.35	1.6469	34.45	1.5372
	12.75	1.1055	25.40	1.4048	54.75	1.7384
	19.65	1.2934	29.40	1.4683	33.05	1.5192
	17.20	1.2355	55.80	1.7466	38.10	1.5809
	12.70	1.1038	36.80	1.5658	60.55	1.7822
Total Weight Gain	149.75	11.4330	327.35	14.8804	476.55	16.6803

$$\sum X = 38.8640$$

$$\sum X^2 = 51.08882$$

$$\sum XY = 56.72323$$

$$\sum Y^2 = 63.64674$$

$$\sum Y = 42.9937$$

$$C = 50.34701$$

$$= 55.69690$$

$$= 61.61527$$

$$\sum x^2 = \underline{0.74181}$$

$$\sum xy = \underline{1.02633}$$

$$\sum y^2 = 2.03147$$

$$b = 1.38$$

2 hrs. at 270°F.

Protein Level	11%		15%		20%	
		(log)		(log)		(log)
Protein Intake (gms) per chicken.	1.180	1.0719	2.014	1.3040	2.817	1.4498
Weekly Weight Gain (gms) per chicken.	8.80	0.9445	13.60	1.1335	47.35	1.6754
	8.95	0.9518	36.15	1.5581	33.45	1.5244
	9.45	0.9754	20.70	1.3160	54.95	1.7400
	7.30	0.8633	14.00	1.1461	54.65	1.7376
	7.85	0.8949	37.40	1.5729	31.15	1.4935
	10.95	1.0394	37.50	1.5740	50.65	1.7046
	11.25	1.0512	35.40	1.5490	55.40	1.7435
	17.30	1.2380	46.40	1.6665	60.35	1.7807
	6.30	0.7993	36.75	1.5653	47.40	1.6758
	12.40	1.0934	39.60	1.5977	43.60	1.6395
Total Weight Gain	100.55	9.8512	317.50	14.6791	478.95	16.7150

$$\sum X = 38.2570$$

$$\sum X^2 = 49.51305$$

$$\sum XY = 53.93446$$

$$\sum Y^2 = 59.75755$$

$$\sum Y = 41.2453$$

$$C = 48.78660$$

$$= 52.59738$$

$$= 56.70582$$

$$\sum x^2 = 0.72645$$

$$\sum xy = 1.33708$$

$$\sum y^2 = 3.05173$$

$$b = 1.84$$

APPENDIX XIII.

Experiment II:

*Analysis of Covariance

Freeze Dried Meat			
$\sum X = 36.7716$	$\sum X^2 = 48.88720$	$\sum XY = 55.22563$	$\sum Y^2 = 63.08317$
$\sum Y = 41.3746$	$C = \underline{48.29110}$	$C = \underline{54.33608}$	$C = \underline{61.13776}$
	$\sum x^2 = 0.59610$	$\sum xy = 0.88955$	$\sum y^2 = 1.94541$
5 minutes at 240°F.			
$\sum X = 36.1296$	$\sum X^2 = 45.83340$	$\sum XY = 53.48809$	$\sum Y^2 = 63.25234$
$\sum Y = 42.2799$	$C = \underline{45.01200}$	$C = \underline{52.67434}$	$C = \underline{61.64103}$
	$\sum x^2 = 0.82140$	$\sum xy = 0.81375$	$\sum y^2 = 1.61131$
Total N = 57			
$\sum X = 72.9012$	$\sum X^2 = 94.72060$	$\sum XY = 108.71372$	$\sum Y^2 = 126.33551$
$\sum Y = 83.6545$	$C = \underline{93.23833}$	$C = \underline{106.99146}$	$C = \underline{122.77325}$
	$\sum x^2 = 1.48227$	$\sum xy = 1.72226$	$\sum y^2 = 3.56226$
Freeze Dried Meat			
$\sum X = 36.7716$	$\sum X^2 = 48.88720$	$\sum XY = 55.22563$	$\sum Y^2 = 63.08317$
$\sum Y = 41.3746$	$C = \underline{48.29110}$	$C = \underline{54.33608}$	$C = \underline{61.13776}$
	$\sum x^2 = 0.59610$	$\sum xy = 0.88955$	$\sum y^2 = 1.94541$
25 minutes at 240°F.			
$\sum X = 34.4253$	$\sum X^2 = 43.26124$	$\sum XY = 49.83310$	$\sum Y^2 = 58.15539$
$\sum Y = 39.5891$	$C = \underline{42.32504}$	$C = \underline{48.67380}$	$C = \underline{55.97488}$
	$\sum x^2 = 0.93620$	$\sum xy = 1.15930$	$\sum y^2 = 2.18051$
Total N = 56			
$\sum X = 71.1969$	$\sum X^2 = 92.14844$	$\sum XY = 105.05873$	$\sum Y^2 = 121.23856$
$\sum Y = 80.9637$	$C = \underline{90.51783}$	$C = \underline{102.93507}$	$C = \underline{117.05572}$
	$\sum x^2 = 1.63061$	$\sum xy = 2.12366$	$\sum y^2 = 4.18284$

* Snedecor, 5th edition.

Freeze Dried Meat			
$\sum X = 36.7716$ $\sum Y = 41.3746$	$\sum X^2 = 48.88720$ $C = \frac{48.29110}{\sum x^2 = 0.59610}$	$\sum XY = 55.22563$ $= \frac{54.33608}{\sum xy = 0.88955}$	$\sum Y^2 = 63.08317$ $= \frac{61.13776}{\sum y^2 = 1.94541}$
2 hrs. at 240°F.			
$\sum X = 38.8640$ $\sum Y = 42.9937$	$\sum X^2 = 51.08882$ $C = \frac{50.34701}{\sum x^2 = 0.74181}$	$\sum XY = 56.72323$ $= \frac{55.69690}{\sum xy = 1.02633}$	$\sum Y^2 = 63.64674$ $= \frac{61.61527}{\sum y^2 = 2.03147}$
Total N = 58			
$\sum X = 75.6356$ $\sum Y = 84.3683$	$\sum X^2 = 99.97602$ $C = \frac{98.63351}{\sum x^2 = 1.34251}$	$\sum XY = 111.94886$ $= \frac{110.02149}{\sum xy = 1.92737}$	$\sum Y^2 = 126.72991$ $= \frac{122.72431}{\sum y^2 = 4.00560}$
Freeze Dried Meat			
$\sum X = 36.7716$ $\sum Y = 41.3746$	$\sum X^2 = 48.88720$ $C = \frac{48.29110}{\sum x^2 = 0.59610}$	$\sum XY = 55.22563$ $= \frac{54.33608}{\sum xy = 0.88955}$	$\sum Y^2 = 63.08317$ $= \frac{61.13776}{\sum y^2 = 1.94541}$
2 hrs. at 270°F.			
$\sum X = 38.2570$ $\sum Y = 41.2453$	$\sum X^2 = 49.51305$ $C = \frac{48.78660}{\sum x^2 = 0.72645}$	$\sum XY = 53.93446$ $= \frac{52.59738}{\sum xy = 1.33708}$	$\sum Y^2 = 59.75755$ $= \frac{56.70582}{\sum y^2 = 3.05173}$
Total N = 58			
$\sum X = 75.0286$ $\sum Y = 82.6199$	$\sum X^2 = 98.40025$ $C = \frac{97.05673}{\sum x^2 = 1.34352}$	$\sum XY = 109.16009$ $= \frac{106.87681}{\sum xy = 2.28328}$	$\sum Y^2 = 122.84072$ $= \frac{117.69048}{\sum y^2 = 5.15024}$

APPENDIX XIV.

Experiment III: Data Plotted for Graph III.

Protein Level	Average Weekly Weight Gain in gms. per chicken.			Daily Protein Intake per chicken in gms.			Log Daily Protein Intake per chicken in gms.		
	11%	15%	20%	11%	15%	20%	11%	15%	20%
Freeze Dried Meat	15.26	36.88	53.60	1.396	1.954	3.210	1.1449	1.2909	1.5065
5 minutes at 240° F.	17.52	36.29	42.98	0.998	2.221	2.347	0.9954	1.3466	1.3705
25 minutes at 240° F.	14.85	32.82	47.11	1.007	1.853	2.772	1.0029	1.2679	1.4428
2 hrs. at 240° F.	14.98	32.74	47.66	1.212	2.204	2.882	1.0835	1.3432	1.4597
2 hrs. at 270° F.	10.06	31.75	47.89	1.180	2.014	2.817	1.0719	1.3040	1.4498

APPENDIX XV.

Experiment III: The *Fiducial Limits of the Regression
Coefficients of the Freeze Dried and
Autoclaved Meats.

The fiducial limits of the regression
coefficient

$$= b - t_{.05} s_b \leq \beta \leq b + t_{.05} s_b$$

Freeze Dried Meat	1.49	±	0.14
5 minutes at 240°F.	0.99	±	0.17
25 minutes at 240°F.	1.23	±	0.17
2 hrs. at 240°F.	1.38	±	0.12
2 hrs. at 270°F.	1.84	±	0.12

* Snedecor, 5th edition.

APPENDIX XVI.

The Growth Rate and Feed Intake Data for
Experiment IV.

Experiment IV:

Meatmeal (Control)

Protein Level	15%		20%	
		(log)		(log)
Protein Intake (gms) per chicken.	1.354	1.1314	2.325	1.3664
Weekly Weight Gain (gms) per chicken.	13.05	1.1155	37.80	1.5775
	6.95	0.8420	25.15	1.4006
	12.65	1.1021	30.05	1.4778
	14.95	1.1747	28.65	1.4572
	7.65	0.8837	34.60	1.5391
	9.45	0.9754	15.50	1.1903
	12.20	1.0864	13.00	1.1139
	11.60	1.0645	28.45	1.4541
	21.65	1.3355	17.90	1.2529
	14.50	1.1614	-	-
Total Weight Gain	124.65	10.7412	231.19	12.4634

$$\begin{aligned}
 \sum X &= 23.6116 & \sum X^2 &= 29.60410 & \sum XY &= 29.18258 & \sum Y^2 &= 29.19411 \\
 \sum Y &= 23.2046 & C &= \frac{29.34251}{\sum X^2} & & = \frac{28.83672}{\sum xy} & & = \frac{28.33965}{\sum y^2} \\
 & & & = 0.26159 & & = 0.34586 & & = 0.85446 \\
 & & & & & & & b = 1.32
 \end{aligned}$$

Meatmeal (Supplemented with L-lysine)

Protein Level	15%		20%	
		(log)		(log)
Protein Intake (gms) per chicken.	1.4583	1.1638	2.533	1.4036
Weekly Weight Gain (gms) per chicken.	19.50	1.2900	29.05	1.4631
	9.15	0.9614	26.15	1.4174
	10.85	1.0354	23.20	1.3655
	28.75	1.4587	37.35	1.5723
	21.55	1.3334	23.75	1.3756
	16.45	1.2161	22.05	1.3434
	16.85	1.2266	31.90	1.5038
	15.05	1.1775	27.60	1.4409
	15.55	1.1917	35.25	1.5471
	17.75	1.2492	-	
Total Weight Gain	171.45	12.1400	256.30	13.0291

$$\sum X = 24.2704$$

$$\sum X^2 = 31.27514 \quad \sum XY = 32.41617 \quad \sum Y^2 = 33.83133$$

$$\sum Y = 25.1691$$

$$C = \frac{31.00275}{\sum x^2 = 0.27239} \quad \frac{32.15074}{\sum xy = 0.26543} \quad \frac{33.34124}{\sum y^2 = 0.49009}$$

$$b = 0.97$$

APPENDIX XVII.

*Analysis of Covariance.

Experiment IV:

Meatmeal (Control)			
$\sum X = 23.6116$	$\sum X^2 = 29.60410$	$\sum XY = 29.18258$	$\sum Y^2 = 29.19411$
$\sum Y = 23.2046$	$C = \frac{29.34251}{\sum x^2 = 0.26159}$	$C = \frac{28.87672}{\sum xy = 0.34586}$	$C = \frac{28.33965}{\sum y^2 = 0.85446}$
Meatmeal (Lysine supplemented)			
$\sum X = 24.2704$	$\sum X^2 = 31.27514$	$\sum XY = 32.41617$	$\sum Y^2 = 33.83133$
$\sum Y = 25.1691$	$C = \frac{31.00275}{\sum x^2 = 0.27239}$	$C = \frac{32.15074}{\sum xy = 0.26543}$	$C = \frac{33.34124}{\sum y^2 = 0.49009}$
Total N = 38			
$\sum X = 47.8820$	$\sum X^2 = 60.87924$	$\sum XY = 61.59875$	$\sum Y^2 = 63.02544$
$\sum Y = 48.3737$	$C = \frac{60.33384}{\sum x^2 = 0.54540}$	$C = \frac{60.95340}{\sum xy = 0.64535}$	$C = \frac{61.57933}{\sum y^2 = 1.44611}$

APPENDIX XVIII.

	Gross Protein Value Data								
	Basal	Casein	Waitara	Wanganui	Fairfield	Ginborne	Belfast	Makarewa	Patea
(1) Mean Weight Gain in 2 weeks (gms)	(Diet No. 1) 13.38	31.84	29.27	30.83	27.52	33.51	29.42	33.77	28.39
(2) Gain in weight over diet 1 (gms)		18.46	15.89	17.45	14.14	20.13	16.04	20.39	15.01
(3) Mean feed consumption in 2 weeks		166.8	154.3	161.7	131.3	182.8	147.3	165.7	163.5
(4) Supplementary protein consumed (gms)		5.0	4.6	4.8	3.9	5.5	4.4	5.0	4.9
(5) Gain in weight over diet 1 per gm supplementary protein.		3.69	3.45	3.63	3.63	3.66	3.64	4.08	3.06
(6) Gross Protein Value [5] X $\frac{100}{3.69}$		<u>100</u>	<u>93</u>	<u>98</u>	<u>98</u>	<u>99</u>	<u>99</u>	<u>111</u>	<u>83</u>

APPENDIX XIX

The Correlation Between the Gross Protein Value and "Available Lysine".

Experiment V:

	Gross Protein Value (Y)	"Available Lysine" (X)
Waitara	93	4.86
Wanganui	98	5.55
Fairfield	98	4.80
Gisborne	99	4.84
Belfast	99	5.51
Makarewa	111	6.50
Patea	83	4.57

$$\sum X = 36.63$$

$$\bar{X} = 5.23$$

$$N = 7$$

$$\sum Y = 681.0$$

$$\bar{Y} = 97.29$$

$$N = 7$$

$$\sum Y^2 = 66,669.00$$

$$\sum XY = 3,591.74$$

$$\sum X^2 = 194.38$$

$$\sum y^2 = \frac{66,252.57}{416.43}$$

$$\sum xy = \frac{3,563.57}{28.17}$$

$$\sum x^2 = \frac{191.68}{2.700}$$

$$r = \frac{\text{Cov. } xy}{\sqrt{\sigma_x^2 \times \sigma_y^2}}$$

$$\sigma_x^2 = 69.500$$

$$\text{Cov. } xy = 4.695$$

$$\sigma_y^2 = 0.451$$

$$r = \frac{4.695}{\sqrt{69.500 \times 0.451}}$$

$$= \underline{0.84} \quad (\text{significant}^*)$$

Experiment V:

Calculation of the Regression Coefficient.

$$(Y - \bar{y}) = b (X - \bar{x})$$

$$(Y - 97.29) = \frac{28.17}{2.7032} (X - 5.23)$$

$$(Y - 97.29) = 10.42 (X - 5.23)$$

$$\underline{Y = 42.79 + 10.42 X}$$

The Fiducial Limits of b

$$s_b^2 = \frac{\sum y^2 - \frac{(\sum xy)^2}{n}}{(n-2) \sum x^2}$$

$$s_b^2 = \frac{4 \cdot 16.43 - \frac{(28.17)^2}{5}}{5 \times 2.70}$$

$$s_b^2 = 9.091$$

$$s_b = \sqrt{9.091} = 3.02$$

° The standard error of b = 10.42 ± 3.02

(t_{.05} for 5 d.f. = 2.571)

° The fiducial limits of b = ± 7.75

APPENDIX XXI

The Correlation between Calcium and Ash.

Ash (X)	Calcium (Y)	
	Determined Values	Estimated Values
20.80	8.12	7.07
4.77	0.80	0.29
32.30	12.68	11.94
19.67	7.06	6.60
24.20	8.60	8.52
21.86	8.10	7.55
12.88	2.79	3.73
3.32	0.56	0.28
16.21	4.24	5.14
19.17	6.07	6.39
19.82	4.60	6.66

$$\sum X = 195.00$$

$$\bar{X} = 17.73$$

$$N = 11$$

$$\sum Y = 63.62$$

$$\bar{Y} = 5.78$$

$$N = 11$$

$$\sum X^2 = 4149.09$$

$$\sum XY = 1420.39$$

$$\sum Y^2 = 500.85$$

$$\underline{3456.82}$$

$$\underline{1127.81}$$

$$\underline{367.95}$$

$$\sum X^2 = 692.27$$

$$\sum xy = 292.58$$

$$\sum y^2 = 132.90$$

$$r = \frac{\text{COV } XY}{\sqrt{b^2_x \times b^2_y}}$$

$$r = \frac{29.268}{\sqrt{69.227 \times 13.29}}$$

$$r = \frac{29.268}{30.33} = 0.96$$

Calculation of the Regression Equation:

$$(Y - \bar{y}) = b (X - \bar{x})$$

$$(Y - 5.78) = \frac{292.58}{692.27} (X - 17.73)$$

$$(Y - 5.78) = 0.423 X - 7.50$$

$$\underline{Y = 0.423 X - 1.72}$$

The Fiducial Limits of b:

$$S_b = \sqrt{\frac{132.90 - \frac{(292.58)^2}{692.27}}{9 \times 692.27}}$$

$$S_b = \sqrt{\frac{9.14}{6230.43}}$$

$$S_b = \sqrt{0.001467}$$

$$S_b = 0.0383$$

∴ The standard error of b = 0.423 ± .0383

($t_{.05}$ for 9 d.f. = 2.262)

∴ The fiducial limits of b = ± 0.0865

The Correlation between Phosphorus and Ash.

Ash (X)	Phosphorus (Y)	
	Determined Values	Estimated Values
20.80	3.58	3.13
4.77	0.31	0.37
32.30	5.29	5.13
19.67	3.66	2.94
24.20	2.03	3.73
21.86	3.45	3.25
12.88	1.20	1.77
3.32	0.11	0.11
16.21	2.25	2.34
19.17	3.46	2.86
19.82	3.37	2.97

$$\sum X = 195.00$$

$$\bar{X} = 17.73$$

$$N = 11$$

$$\sum Y = 28.71$$

$$\bar{Y} = 2.61$$

$$N = 11$$

$$\sum X^2 = 4149.09$$

$$\sum XY = 628.76$$

$$\sum Y^2 = 100.16$$

$$\underline{3456.82}$$

$$\underline{508.95}$$

$$\underline{74.93}$$

$$\sum x^2 = 692.27$$

$$\sum xy = 119.81$$

$$\sum y^2 = 25.23$$

$$r = \frac{\text{COV } XY}{\sqrt{\sigma_x^2 \times \sigma_y^2}}$$

$$r = \frac{11.981}{\sqrt{69.227 \times 2.523}}$$

$$r = \frac{11.981}{13.22} = 0.91$$

The Calculation of the Regression Equation:

$$(Y - \bar{y}) = b (X - \bar{x})$$

$$(Y - 2.61) = \frac{119.81}{692.27} (X - 17.73)$$

$$(Y - 2.61) = 0.173 X - 3.07$$

$$\underline{Y = 0.173 X - 0.46}$$

The Fiducial Limits of b:

$$s_b = \sqrt{\frac{25.23 - \frac{(119.81)^2}{692.27}}{9 \times 692.27}}$$

$$s_b = \sqrt{\frac{4.49}{6230.43}}$$

$$s_b = \sqrt{0.00072}$$

$$s_b = 0.0268$$

∴ The standard error of b = 0.173 ± 0.027

(t_{.05} for 9 d.f. = 2.262)

∴ The fiducial limits of b = ± 0.0715