Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
THE NUTRITIONAL VALUE OF RYEGRASS - WHITE CLOVER

LEAF PROTEIN CONCENTRATE

A thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry at Massey University.

DAVID CARVILLE JOHNS

1983
ERRATA

Incorrect Data
1. The energy intake values presented in Table VI p. 23 should read as 7218.0, 6079.8, 7550.9 and 6043.2 respectively and the energy values present in Table VIII p. 27 should read 9304.9, 9445.6, 9313.4 and 8384.2. The amount of cornstarch added in Diet I, presented in Table XIII, Appendix A, p. 167 should read 4.20.

Typographical and Editing Errors
1. p. 66 Second paragraph - the phrase "soybean enzyme" should read "soybean enzyme inhibitor".
2. p. 163, Table IX Appendix A: The phrase "average phosphorus" should read "available phosphorus."
3. p. 196, Table XVI, Appendix B: The phrase "trypsinogen and chymotrypsinogen levels" should read "trypsin and chymotrypsin activities."
4. pp. 155,156,161: The words lupin or lupins refer to lupin meal.
5. p. 154: The title "Ruakura Animal Research Centre" should read "Ruakura Agricultural Research Centre."
8. The following typographical/spelling errors should be noted:
   - compative (comparative) p. 53
   - titerperoids (triterpenoids) p. 67
   - nutrional (nutritional) p.70
   - intern (interim) p. 134
   - proteolytic (proteolytic) p. 143
   - Tokyo (Tokyo) p. 154
   - lysine (glycine) p. 155, 158
   - mammalian (mamalian) p. 204
   - Oldfield (Oldfield) p. 69
   - Scheline (Scheline) p. 81
9. p. (11) The phrase "... estimates for the LPC diet was ..." should read "... estimates for the LPC diet were ...
10. The following headings should read:
   - p. 52 Effect of Pelleting, Methionine on Increasing Inclusion Levels of LPC (15%, 20%) on Chick Growth.
   - p. 54 Effect of Pelleting, Methionine Supplementation and of Different Levels of LPC (15%,20%) on Chick Growth.
   - p. 113 Comparison of the "Corrected"(CAAA) and Apparent (ApAAA) Amino Acid Availability of LPC and SBM Ingredients and of Diets Containing LPC and SBM.
   - p. 123 Comparison of Amino Acid Digestibility Measured at the Ileum with Amino Acid Availability Measured by Excreta Analysis.
11. The following words should read:
   - p-Nitroaniline (p-nitroaniline) p. 75
   - Inhibitor (inhibitor) p. 144
   - Cornstarch (cornstarch) p. 154
12. The symbol (G)* should be included on Table VII, p. 26 and Table IX, p. 32. Also the symbol (g) should be included on Table XXVI, p.131.

1084007044
ABSTRACT

Leaf Protein Concentrate (LPC), which was manufactured from a mixture of Ryegrass and White Clover (Lolium perenne and Trifolium repens) at the Ruakura Agricultural Research Centre, was evaluated as a possible protein source for feeding to chickens. The nutritional value of LPC was compared to that of soybean meal (SBM).

LPC was shown to have a lower nutritional value than SBM in the growth trials. The addition of methionine or cystine to the diet containing LPC improved both food utilisation and weight gain of the chickens. These growth parameters showed the greatest improvement when 2 g methionine/kg diet was added to the LPC diet. The additional quantity of 2 g methionine/kg diet was similar to the amount of sulphur amino acid contributed by LPC to the diet; 1.8 g sulphur amino acid from LPC/kg diet. When an equivalent amount of cystine (1.6 g) to methionine, on a sulphur basis, was added to the LPC diet and fed to chickens, it was shown to support the same amount of growth and maintain a similar food utilisation level as additional methionine. LPC contributed only 0.6 g cystine/kg of diet. As this was much lower than the added cystine and/or methionine, it was concluded that the availability of cystine in the whole diet was reduced by the presence of LPC rather than the lack of availability of cystine in LPC alone.

The following information was also obtained:-

(i) Pancreatic hypertrophy and increased pancreatic enzyme activity (trypsin and chymotrypsin) occurred due to feeding the LPC diet.

(ii) The in vitro exhaustive enzyme digestibility study indicated that while the overall digestibility of LPC was approximately 6% lower than that of SBM, none of the individual amino acid digestibility estimates in LPC diverged markedly from the mean. All LPC amino acids were released equally by enzyme hydrolysis.
(iii) In contrast to the in vitro findings, the in vivo mean amino acid availability estimates for the ingredient LPC (as measured in the excreta) were lower than the corresponding SBM estimates by approximately 15%. The cystine availability estimate for the ingredient LPC was only 51.2% in terms of corrected amino acid availability (CAAA), and 11.9% in terms of apparent amino acid availability (ApAAA). By comparison the cystine availability estimates for the ingredient SBM were 80.8% CAAA and 75.7% ApAAA. When the diets containing LPC or SBM were assayed by the same technique, the differences in the amino acid availability estimates were markedly reduced. The availability estimates of cystine in the LPC diet were still lower than the other amino acid availability estimates for the LPC diet. These however were only 8-10% lower than the corresponding estimates for the SBM diet.

(iv) The mean amino acid digestibility estimates, derived by analysis of the ileal contents of chickens fed with the LPC diet were 26% lower than those for chickens fed the SBM diets. The cystine digestibility estimates for the LPC diet was approximately 45% lower than the corresponding cystine digestibility estimate for the SBM diet. These results indicated that digestion and/or absorption of the LPC diet was probably being retarded as compared with the SBM diet.

(v) Supplementation of the LPC diet with the antibiotic, Neomix, gave an improvement in growth and an increase in the mean amino acid availability (measured by excreta analysis) of approximately 7%. This indicated that the gut microflora were influencing the nutritional value of LPC.

Feeding the LPC diet in comparison to feeding the SBM diet also tended to increase the level of C19 cyclopropane fatty acid in the excreta. This indicated that feeding the LPC diet was influencing the nature and/or activity of the microfloral population.
The physiological and metabolic effects of feeding raw soybean meal and/or trypsin inhibitors, which have been reported in the literature, included pancreatic hypertrophy, increased pancreatic proteolytic enzyme activity, retardation of ileal protein digestibility and an influence by gut microflora. Each of these factors were characteristic of chickens fed the LPC diet. It was therefore concluded that the additional need for cysteine or methionine by chickens fed the LPC diet, was due to the presence of trypsin inhibitors in the LPC.

It was demonstrated, by feeding L-(methyl $^{14}$C) methionine that phenolic compounds were being methylated. However the need for detoxification of aromatic compounds, which required methionine (as a methyl donor) and/or arginine (ornithine), could not explain the growth depression experienced by chickens fed the unsupplemented LPC diet.

The feeding of L-(methyl $^{14}$C) methionine in conjunction with the LPC diet also indicated that the digestibility of methionine was not being hindered during the digestive process by preferential binding with other compounds in the LPC diet.

It was concluded from the results of this study that LPC adequately supplemented with methionine, could be a useful addition to the range of ingredients available for use in poultry feeds.
ACKNOWLEDGEMENTS

I would like to thank my Supervisors Dr. M.N. Wilson and Mr. M.R. Patchell for their interest and co-operation during this study. In particular I wish to express my appreciation to Dr. S. Bornstein for his encouragement during the early stages of this study and to Dr. D.R. Husbands and Dr. J.C. Hawke for their helpful advice and assistance.

I am also indebted to Dr. P.E. Donnelly and his team at Ruakura Animal Research Centre for supplying the Leaf Protein Concentrate and to the Poultry Research Centre staff for their help in both the management of the trials and in the analysis of the amino acids.

Special thanks is also given to my wife for all her work typing this thesis and for the encouragement given.
TABLE OF CONTENTS

SECTION 1

Chapter 1: Introduction ........................................... 1
           Leaf Protein Concentrate .......................... 2

Chapter 2: Materials and Methods for Growth Trials ..... 9
           a) Leaf Protein Concentrate ......................... 9
           b) Diets Used to evaluate the nutritional value of LPC ..................... 11
           c) Chickens ........................................ 14
           d) Management ..................................... 15
           e) Chemical Analysis ............................... 16
           f) Determination of Metabolizable Energy ............. 17
           g) Statistical Analysis ............................ 19

Chapter 3: Growth Trials - Part I
           Preliminary Growth Trials .......................... 20
           Preliminary Trial 1 .................................. 20
           Results ............................................. 21
           Preliminary Trial 2: Lysine and Methionine Supplementation ............. 25
           Results ............................................. 25
           Preliminary Trial 3: Lysine and Methionine Supplementation cont. ......... 30
           Results ............................................. 31
           Discussion (Trials 1-3) ................................ 35

Chapter 4: Growth Trials - Part II
           Amino Acid Supplementation of LPC Diets ............. 39
           Trial 4 - Effect of Additional Lysine, Arginine or Methionine ............. 40
           Results ............................................. 41
           Trial 5: Methionine and Arginine Supplementation ..................... 44
           Results ............................................. 46
Chapter 4:  Growth Trials - Part II continued:

<table>
<thead>
<tr>
<th>Trial</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Methionine and Cystine Supplementation</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Results</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Effect of Pelleting and Methionine Supplementation on Increasing Inclusion Levels of LPC (15,20%) on Chick Growth</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Results</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Discussion (Trials 4-7)</td>
<td>58</td>
</tr>
</tbody>
</table>

SECTION II:

Chapter 5:  Introduction - Some Compounds Influencing the Nutritional Value of LPC

1. Phenolic Compounds
   (a) Detoxification
   (b) Formation of Complexes
   (c) Enzyme Inhibition

2. Protein-Carbohydrate Interaction
3. Formation of Oxides of Methionine
4. Oxidation of Unsaturated Fatty Acids
5. (i) Saponins
   (ii) Oestrogenic substances

Discussion

Chapter 6:  Methionine and Arginine - Detoxification of Aromatic Compounds Present in LPC

I Laboratory Techniques (Summary)
II Experiments (Summary)

I - Laboratory Techniques
   (a) Paper and Thin Layer Chromatography
   (b) High Voltage Electrophoresis
   (c) Determination of Radioactivity
   (d) Stains
      Phenolic Identification: Sulphanilic Acid and p-Nitroaniline stains
      Prussian Blue
      Protein/Amino Acid Identification:Ninhydrin
## Chapter 6: Methionine and Arginine - continued:

<table>
<thead>
<tr>
<th>Process</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid Identification: 2',7'Dichlorofluorescein</td>
<td>76</td>
</tr>
<tr>
<td>(e) Gas Liquid Chromatography</td>
<td>77</td>
</tr>
<tr>
<td>Methylation of Fatty acids</td>
<td>77</td>
</tr>
<tr>
<td>Hydrogenation</td>
<td>77</td>
</tr>
<tr>
<td>Bromination</td>
<td>78</td>
</tr>
<tr>
<td>Collection of Radioactive Effluent</td>
<td>78</td>
</tr>
</tbody>
</table>

## Experiments

**Experiment 1: "Does Methylation of Phenolic Compounds Occur?"

1. (a) Separate Collection of Faeces and Urine...
2. (b) Initial Urine Extraction and Chromatography...
3. (c) Excreta Extraction...
4. (d) Initial feeding of L-(methyl $^{14}$C) Methionine...
5. (e) Initial Identification of $^{14}$C Labelled Compounds...
6. (f) Secondary Identification of $^{14}$C Labelled Compounds...

(A) Lower Phase ("Lipid Fraction") Analysis...
   1. (a) Analysis of the presence of $^{14}$C in phospholipids...
   2. (b) Test for Presence of $^{14}$C in the Free Fatty Acids - mono-, di-, tri-glycerides...
   3. (c) Methylation...
   4. (d) Test for Degree of Saturation...
   5. (e) T.L.C. of Methyl Esters...

(B) Upper Phase ("Water Fraction") Analysis...

**Experiment 2: "Is the Excretion Level of the Methyl Group From Methionine Higher Due to Feeding LPC?"

**Method**

1. (a) Treatments...
2. (b) Preparation of Excreta for Total $^{14}$C Count...
3. (c) Fractionation...

**Results**
Chapter 6: Methionine and Arginine - continued:

Experiment 3: Measurement of the Ornithine Excretion Level Due to Feeding LPC and SBM

Method ................................................. 107
Results .................................................. 108
Discussion (Experiments 1-3) ......................... 110

Chapter 7: Factors Influencing Digestion

Experiment 4: Exhaustive Enzyme Digestion of "Pure" LPC and SBM

Method ................................................... 114
(i) Enzymes .............................................. 114
(ii) Buffer ................................................ 114
(iii) Exhaustive Digest ................................. 114
Results .................................................... 115

Experiment 5: Comparison of the "Corrected (CAAA) and Apparent (ApAAA) Amino Acid Availability of LPC and SBM Ingredients and of the Diets Containing LPC and SBM."

Method ................................................... 117
Results .................................................... 118

Experiment 6: Comparison of Amino Acid Digestibility Measured at the Ileum with Amino Acid Availability Measured by excreta analysis...

Method ................................................... 123
Results .................................................... 124

Experiment 7: Effect of LPC on Pancreatic Size and Level of Pancreatic Proteolytic Enzymes

Method (i) Treatment ................................... 127
(ii) Pancreatic Enzymes ............................... 127
Results .................................................... 129
**Chapter 7:** Methionine and Cystine - The Presence of Trypsin Inhibitors continued:

<table>
<thead>
<tr>
<th>Experiment 8: Effect of Antibiotics on the Apparent Amino Acid Availability in LPC or SBM Diets as Measured in the Excreta</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>130</td>
</tr>
<tr>
<td>Results</td>
<td>130</td>
</tr>
</tbody>
</table>

**Discussion (Experiments 4-8):**

- (i) Digestibility and/or Availability Studies: 134
- (ii) Pancreatic Hypertrophy and Enzyme Levels: 136
- (iii) Contributory Effect of the Gut Microflora: 137

**Chapter 8:** Discussion: 146

**Appendices:**

- Appendix A: 154
- Appendix B: 176

**Bibliography:** 199
## LIST OF TABLES

Table I: The Lysine and Isoleucine Content of Various Feed Ingredients (Bornshtein, 1977) ........................................ 3

Table II: The Amino Acid Content \(\text{[g (amino acid)/16 g N]}\) of Leaf Protein Concentrate (LPC) and Soybean Meal (SBM). ........................................ 5

Table III: Analysis of Leaf Protein Concentrates Supplied During the Period of this Study..................12

Table IV: Analysis of Soybean Meals Supplied During the Period of this Study. ........................... 13

Table V: Initial Evaluation of LPC in Comparison to SBM (days 0 to 28). ................................. 22

Table VI: Summary of Amino Acid Intakes and Energy Intakes in Trial 1. ........................................ 23

Table VII: Trial 2 – Effect on Growth of Supplementation of LPC with 0.1% Lysine + 0.03% Methionine and with an Additional 5% LPC, in Comparison with SBM (1-28 days) ........................................ 26

Table VIII: Amino Acid and Energy Intakes in Trial 2 ................................................ 27

Table IX: Trial 3 – Effect on Growth of Supplementation of LPC with 0.15% Lysine + 0.05% Methionine or with SBM, and the Effect of Pelleting in Comparison with SBM (days 0 to 28) ........................................ 32

Table X: Amino Acid Intakes in Trial 3 ........................................ 33

Table XI: Summary of Trials 1-3: Amino Acids in the LPC Diet which have intakes below SBM Diet intakes and “NRC Equivalent Level.” ........................................ 37

Table XII: Ranking of LPC Amino Acid Intake Compared to “70% NRC equivalent Level” in Trials 1-3 (Comparison at different levels of LPC). ........................................ 38

Table XIII: Trial 4 – Effect of Supplementing LPC Diets with Arginine, Lysine and Methionine ............ 42

Table XIV: Trial 5 – Effect of Supplementing LPC Diets with Methionine and Arginine .................. 45

Table XV: Trial 6 – Results of Cystine Supplementation (21 days). ........................................ 50

Table XVI: Effect of Pelleting and Methionine Supplementation on Increasing Inclusion Levels of LPC (15,20%) on Chick Growth (21 days) ........................................ 54
Table XVII: The Distribution of Radioactivity after Methylation .................. 95
Table XVIII: The Distribution of Radioactivity after Hydrogenation and Bromination.................. 96
Table XIX: Comparison of the Effect of LPC and Antibiotic with that of SBM and Antibiotic on the Level of Methyl Group ¹⁴C Excretion. ................................. 106
Table XX: Summary of Analysis for Ornithine Excretion Level and the Effect of Methionine and Arginine Supplementation ................................. 109
Table XXI: Results of in vitro Exhaustive Digestion. .................. 116
Table XXII: Summary of the Results of Experiment 5 - LPC and SBM Ingredient Amino Acid Availability using the THE Method ................................. 120
Table XXIII: Summary of the Results of Experiment 5 - LPC and SBM Dietary Amino Acid Availability using the THE Method ................................. 121
Table XXIV: Summary of the Results of Experiment 6 - Comparison of the Apparent Amino Acid Digestibility (ApAAD)/Availability (ApAAA) as Measured in the Ileum and Excreta. ................................. 125
Table XXV: Effect of Feeding LPC on Pancreatic Size and Level of Pancreatic Proteolytic Enzymes (Experiment 7). ................................. 128
Table XXVI: Experiment 8: Effect of Antibiotic (Neomix) Supplementation on the Growth of Chickens fed LPC and SBM Diets................................. 131
Table XXVII: The Effect of Antibiotics on Apparent Amino Acid Availability (Total collection of excreta in Experiment 8) in LPC or SBM Containing Diets ................................. 132
Table XXVIII: Availability of Sulphur Amino Acids (Cystine and Methionine) in LPC and SBM and in diets containing LPC and SBM as Determined by Several Methods. ................................. 141
LIST OF FIGURES

Fig. 1: Extraction and Processing System Used at the Ruakura Agricultural Research Centre (Donnelly, 1980) ........................................ 10

Fig. 2: The Effect of Methionine and Arginine Supplementation on Weight Gain of Chicks Fed LPC Diets (Trial 5) ................................. 47

Fig. 3: The Effect of Methionine and Arginine Supplementation on Food Utilisation of 15% LPC Diets (Trial 5) ................................. 47

Fig. 4: Effect of Pelleting and Methionine Supplementation on Weight Gain with Two Levels of LPC Compared to Equivalent SBM Levels (Trial 6) ................................. 55

Fig. 5: Effect of Pelleting and Methionine Supplementation on Food Utilisation With Two Levels of LPC Compared to Equivalent SBM Levels (Trial 6) ................................. 56

Fig. 6: Enzyme-Catalyzed Oxidation of Caffeic Acid to Caffeoylquinone Followed by Autolytic Bonding to Amino and Thiol Groups in Proteins (Sosulski, 1979) ................................. 63

Fig. 7: Proposed Scheme for the Metabolic Transformations of Caffeic Acid (from Booth et al., 1957) ................................. 80

Fig. 8: Metabolism of Protochatechuic Acid and Homoprotochatechuic Acid (from Parke, 1968) ................................. 83

Fig. 9: Two-dimensional Schematic Chromatogram of the "Urine Ether Fraction". The Coloured Reactions of the Spots with Suphanilic Acid and p-Nitroaniline are Shown Below. The Position of the Standards used is also Shown. ................................. 85

Fig. 10: One Dimensional Development of the "Ether Excreta" Fraction Using Various Developing Solvents ................................. 88

Fig. 11: One Dimensional Development of the "Ether Excreta" Fraction Using Various Developing Solvents ................................. 89

Fig. 12: Test for $^{14}$C in the Phospholipids ................................. 91

Fig. 13: Test for Presence of $^{14}$C in the Free Fatty Acids ................................. 92

Fig. 14: The Effect of Methylation on the Movement of the Radioactive Fraction ................................. 93
<table>
<thead>
<tr>
<th>List of Figures continued:</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. 15: The Movement of the Radioactivity ($^{14}$C) on Silver Nitrate TLC plates</td>
<td>94</td>
</tr>
<tr>
<td>Fig. 16: Two Dimensional Development (&quot;BAW&quot;; 2% HAc) of the &quot;Water Fraction&quot; Before and After Acid Hydrolysis</td>
<td>100</td>
</tr>
<tr>
<td>Fig. 17: The Results of the Two Stage Determination to Test Whether the Unhydrolysed &quot;Water Fraction&quot; contained Phenolic and Protein Material Associated Together</td>
<td>102</td>
</tr>
<tr>
<td>Fig. 18: Regulation of Trypsin Secretion (Adapted from Anderson et al., 1979)</td>
<td>144</td>
</tr>
<tr>
<td>Fig. 19: Relationship between the SBM and LPC Dietary Amino Acid Digestibility as Measured in the Ileum</td>
<td>146</td>
</tr>
<tr>
<td>Fig. 20: Scheme to Explain the Possible Deleterious Effect of Proteinase Inhibitors on the Nutritive Value of Protein (Modification of Richardson, 1981)</td>
<td>150</td>
</tr>
<tr>
<td>Fig. 21: Relationship Between Weight Gain and Organic Sulphur/Phenolic Intake (data calculated from Subba Rau et al., 1972)</td>
<td>152</td>
</tr>
<tr>
<td>Fig. 22: Relationship Between Weight Gain and Cystine Sulphur/Phenolic Intake (data calculated from Subba Rau et al., 1972)</td>
<td>153</td>
</tr>
</tbody>
</table>
APPENDIX A: Summary of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table I</td>
<td>Constraints used in the Formulation of Diets for Trial 1</td>
<td>155</td>
</tr>
<tr>
<td>Table II</td>
<td>The Ingredient Composition of the Experimental Diets used in Trial 1</td>
<td>156</td>
</tr>
<tr>
<td>Table III</td>
<td>The Nutrient Composition of the Experimental Diets Used in Trial 1</td>
<td>156</td>
</tr>
<tr>
<td>Table IV</td>
<td>Constraints used in the formulation of Diets for Trial 2</td>
<td>157</td>
</tr>
<tr>
<td>Table V</td>
<td>Ingredient Composition of the Experimental Diets - Trial 2</td>
<td>158</td>
</tr>
<tr>
<td>Table VI</td>
<td>The Nutrient Composition of Rations Used in Trial 2</td>
<td>159</td>
</tr>
<tr>
<td>Table VII</td>
<td>Constraints used in the Formulation of Diets for Trial 3</td>
<td>160</td>
</tr>
<tr>
<td>Table VIII</td>
<td>Ingredient Composition of Experimental Diets, Trial 3</td>
<td>160</td>
</tr>
<tr>
<td>Table IX</td>
<td>The Nutrient Composition of Rations used in Trial 3</td>
<td>161</td>
</tr>
<tr>
<td>Table X</td>
<td>Constraints used in the Formulation of the Basal for Trials 4-7</td>
<td>162</td>
</tr>
<tr>
<td>Table XI</td>
<td>The Calculated Composition of the Basal used in Trials 4-7</td>
<td>163</td>
</tr>
<tr>
<td>Table XII</td>
<td>The Ingredient Composition of the Basal Used in Trials 4-7</td>
<td>164</td>
</tr>
<tr>
<td>Table XIII</td>
<td>Ingredient Composition of Diets Used in Trial 4</td>
<td>165</td>
</tr>
<tr>
<td>Table XIV</td>
<td>The Calculated Nutrient Composition of Diets Used in Trial 4</td>
<td>166</td>
</tr>
<tr>
<td>Table XV</td>
<td>Ingredient Composition of Diets Used in Trial 5</td>
<td>167</td>
</tr>
<tr>
<td>Table XVI</td>
<td>The Calculated Nutrient Composition of the Diets Used in Trial 5</td>
<td>168</td>
</tr>
<tr>
<td>Table XVII</td>
<td>Ingredient Composition of Diets Used in Trial 6</td>
<td>169</td>
</tr>
<tr>
<td>Table XVIII</td>
<td>The Calculated Nutrient Composition of Diets Used in Trial 6</td>
<td>170</td>
</tr>
<tr>
<td>Table XIX</td>
<td>Ingredient Composition of Diets Used in Trial 7</td>
<td>171</td>
</tr>
</tbody>
</table>
**APPENDIX A: Summary of Tables continued:**

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table XX:</td>
<td>The Nutrient Composition of Diets Used in Trial 7.</td>
<td>173</td>
</tr>
<tr>
<td>Table XXI:</td>
<td>Ingredient Composition of Diets Used in Experiment 2.</td>
<td>174</td>
</tr>
<tr>
<td>Table XXII:</td>
<td>Amino Acid Composition of the LPC and SBM Diets.</td>
<td>174</td>
</tr>
<tr>
<td>Table XXIII:</td>
<td>Ingredient Composition of Diets Used in Experiment 5.</td>
<td>175</td>
</tr>
<tr>
<td>Table XXIV:</td>
<td>Amino Acid Composition of Diets Used in Experiment 5.</td>
<td>175</td>
</tr>
</tbody>
</table>
APPENDIX B: Summary of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Analysis of Variance - Trial</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>178</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>179</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>180</td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>181</td>
</tr>
<tr>
<td>V</td>
<td>3</td>
<td>182</td>
</tr>
<tr>
<td>VI</td>
<td>3</td>
<td>183</td>
</tr>
<tr>
<td>VII</td>
<td>4</td>
<td>184</td>
</tr>
<tr>
<td>VIII</td>
<td>5</td>
<td>185</td>
</tr>
<tr>
<td>IX</td>
<td>6</td>
<td>186</td>
</tr>
<tr>
<td>X</td>
<td>7</td>
<td>187</td>
</tr>
<tr>
<td>XI</td>
<td>2</td>
<td>189</td>
</tr>
<tr>
<td>XII</td>
<td>3</td>
<td>190</td>
</tr>
<tr>
<td>XIII</td>
<td>5</td>
<td>191</td>
</tr>
<tr>
<td>XIV</td>
<td>5</td>
<td>193</td>
</tr>
<tr>
<td>XV</td>
<td>6</td>
<td>195</td>
</tr>
<tr>
<td>XVI</td>
<td>7</td>
<td>196</td>
</tr>
<tr>
<td>XVII</td>
<td>8</td>
<td>197</td>
</tr>
<tr>
<td>XVIII</td>
<td>8</td>
<td>198</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AME</td>
<td>Apparent Metabolisable Energy</td>
</tr>
<tr>
<td>ApAAA</td>
<td>Apparent Amino Acid Availability</td>
</tr>
<tr>
<td>ApAAD</td>
<td>Apparent Amino Acid Digestibility</td>
</tr>
<tr>
<td>Arginine</td>
<td>L-Arginine (used to supplement diets)</td>
</tr>
<tr>
<td>CAAA</td>
<td>Corrected Amino Acid Availability</td>
</tr>
<tr>
<td>Histidine</td>
<td>L-Histidine (used to supplement diets)</td>
</tr>
<tr>
<td>Lysine</td>
<td>L-Lysine. HCl (used to supplement diets)</td>
</tr>
<tr>
<td>LPC</td>
<td>Leaf Protein Concentrate</td>
</tr>
<tr>
<td>Methionine</td>
<td>DL-methionine (used to supplement diets)</td>
</tr>
<tr>
<td>N.S.</td>
<td>Not Significant</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinyl pyrollidone</td>
</tr>
<tr>
<td>SBM</td>
<td>Soybean meal</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-layer Chromatography</td>
</tr>
<tr>
<td>TME</td>
<td>True Metabolisable Energy</td>
</tr>
</tbody>
</table>