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.

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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 L presented in Table XIII, Appendix A, p. 167 should read 4.20.

Typographical and Editing Errors

1. p. 65 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. 155,158,161 : The words lupin or lupins refer to lupin meal.

5. p. 154: The title "Ruakura Animal Research Centre" should read "Ruakura Agricultural Research Centre."

6. The reference Heywang, B.,C.R. Thompson and A.R. Kemmerer (1957) Poult. Sci., 38: 968 should read Heywang, B., C.R. Thompson and A.R. Kemmerer (1959) Poult. Sci., 38:968.

7. p. 199 The reference to Bondi et al. (1973) should read: Bondi, A., Y. Birk and B. Gestetner (1973) In: Chemistry and Biochemistry of Herbage (ed. Butler, G.W. and R.W. Bailey) Vol. 1, Academic Press, London, p. 511.

8. The following typographical/spelling errors should be noted:
   comparitive (comparative) p. 53
   titerperoids (triterpenoids) p. 67
   nutrtional (nutritional) p.70
   interum (interim) p. 134
   proteloytic (proteolytic) p. 143
   Tokoyo (Tokyo) p. 154
   glysine (glycine) p. 155, 158
   mamalian (mammalian) p. 204
   Olfield (Oldfield) p. 69
   Schleine (Scheline) p. 81

9. p. (ii) The phrase " ... estimates for the LPC diet was ..." should read ".... estimates for the LPC diet were ..."

   p. 21 The sentence "The results of Trial 1 were summarised in Table V." should read "The results of Trial 1 are summarised in Table V."

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.

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 C_{19} 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 cystine 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

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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.

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LIST OF ABBREVIATIONS

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