



**UNIVERSITI PUTRA MALAYSIA**

**NUTRITIVE VALUE AND BIOAVAILABILITY OF PROTEIN  
FEEDSTUFFS FOR BROILER CHICKENS**

**FAIRDA LIM**

**FPV 1985 2**

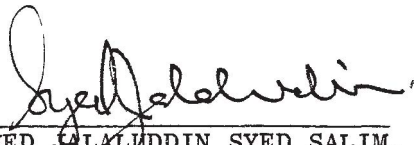
This is to certify that we have read this thesis entitled "Nutritive Value and Bioavailability of Protein Feedstuffs for Broiler Chickens" by Fairda Lim, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirements for the degree of Master of Science.



AZANG F. ZAINUDDIN, Ph.D.  
Assoc. Professor/Dean of Graduate Studies  
Universiti Pertanian Malaysia  
(Chairman Board of Examiners)



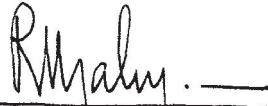
I.R. SIBBALD, D.Sc.  
Chairman, Poultry Nutrition Program  
Animal Research Centre  
Canada  
(External Examiner)



SYED JALALUDDIN SYED SALIM, Ph.D.  
Professor/Deputy Vice-Chancellor  
Universiti Pertanian Malaysia  
(Internal Examiner)



P. VIJCHULATA, Ph.D.  
Lecturer  
Faculty of Veterinary Medicine & Animal Sciences  
Universiti Pertanian Malaysia  
(Internal Examiner)



R. I. HUTAGALUNG

Profesor

Department of Animal Science  
Faculty of Veterinary Medicine & Animal Sciences  
Universiti Pertanian Malaysia  
(Supervisor)

This thesis was submitted to the Senate of Universiti Pertanian Malaysia and was accepted as partial fulfilment of the requirements for the degree of Master of Science.

Date: 14 NOV 1985



ALANG. P. ZAINUDDIN, Ph.D.  
Associate Professor/  
Dean of Graduate Studies

NUTRITIVE VALUE AND BIOAVAILABILITY OF PROTEIN FEEDSTUFFS  
FOR BROILER CHICKENS

THESIS

A thesis submitted in partial fulfillment of the  
requirements for the Degree of Master of Science  
at Universiti Pertanian Malaysia

by

FAIRDA LIM

Faculty of Veterinary Medicine and Animal Science  
Universiti Pertanian Malaysia  
Serdang, Selangor, Malaysia  
1985



## ACKNOWLEDGEMENT

The author expresses her sincere gratitude and appreciation to her supervisor, Professor Dr. Rudy I. Hutagalung, for his valuable supervision and guidance in the planning and implementation of experiments and the preparation of dissertation, and Dr. Yeong Shue Woh, as a consultant and the provision of facilities during the course of experimental work.

The author is also grateful to the Director-General of MARDI for providing scholarship and study leave to undertake the higher degree programme at the Universiti Pertanian Malaysia.

Sincere thanks are extended to Dr. V. Rhagavan of the Federal Flour Mill for the supply of FFM soybean meal and Mr. Sunny Heng Kee Siang, the Manager for Export and Development Division, Industrial Business Area, I.C.I. (Malaysia) Sdn. Bhd. for the supply of "Pruteen" for the experiments.

Special gratitude and appreciation is due to Mr. Ong Hwee Keng for his motivation throughout the programme.

Special thanks are also extended to the Staff of MARDI, including all personnel in the Animal Nutrition Laboratory, Mr. Ahmad Shukri bin Haji Othman for the analysis of data and Mr. Ong Boon Thuan for his amino acid analyses.

Last but not least, appreciation is also due to Dr. C. Devendra for his encouragement.



## TABLE OF CONTENTS

	Page
TITLE	i
ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF APPENDIX TABLES	viii
LIST OF ABBREVIATIONS	x
ABSTRACT	xi
CHAPTER I INTRODUCTION	1
CHAPTER II LITERATURE REVIEW	
METHODS OF FEED EVALUATION	3
1. Chemical evaluation	3
(i) Proximate analysis	3
(ii) Gross energy	6
(iii) Minerals	6
(iv) Fibre analysis	6
(v) Amino acid	8
(vi) <u>In vitro</u> evaluation	8
2. Feeding values of proteinaceous feedstuffs	9
Comparative nutritive values of some protein sources	10
Fish meal	10
Soybean meal	11
Pruteen	12



	Page
3. Biological evaluation	13
(i) Bioavailable energy and determination	13
Apparent Metabolizable Energy (AME)	14
True Metabolizable Energy (TME)	15
Indirect measurement of ME	16
Selection of a bioassay	16
(ii) Amino acid availability (AAA) and determination	17
Biological assays	19
Enzymatic assays	21
Chemical assays	21
Microbiological assays	22
Faecal and ileal assays	23
AAA from TME determination	24
 CHAPTER III EXPERIMENT 1	
Introduction	25
Materials and Methods	25
Results	26
Discussion	32
 CHAPTER IV EXPERIMENT 2	
Introduction	35
Experiment 2a	35
Materials and methods	35
Results	40
Discussion	47
Experiment 2b	52





	Page
Materials and methods	52
Results	55
Discussion	61
CHAPTER V EXPERIMENT 3	
Introduction	64
Materials and methods	64
Results	67
Discussion	71
CHAPTER VI GENERAL DISCUSSION	76
CHAPTER VII SUMMARY AND CONCLUSION	78
BIBLIOGRAPHY	79
APPENDICES Appendix 1	A1
Appendix Tables	A4



## LIST OF TABLES

TABLE		PAGE
1	Chemical composition of protein sources	27
2	Amino acid profiles of protein sources	30
3	<u>In vitro</u> digestibility of proteinaceous feedstuffs	31
4	Composition of starter diets in Experiment 2a	37
5	Composition of finisher diets in Experiment 2a	38
6.	Effects of fish meals and Pruteen on the performance of broiler chickens (8 weeks)	44
7	Effects of dietary levels of fish meals and Pruteen on apparent digestibilities of the starter diets by broiler chickens	45
8	Composition of starter diets in Experiment 2b	53
9	Composition of finisher diets in Experiment 2b	54
10	Effects of level and type of soybean meals on the performance of broiler chickens (8 weeks)	59
11	Effects of dietary level and type of soybean meals on apparent digestibilities of the starter diets by broiler chickens	60
12	True metabolizable energy (TME) and nitrogen-corrected metabolizable energy (TMEn) values	68
13	Amino acid availability (% DM) of proteinaceous feedstuffs in chickens	69
14	Amino acid and true available amino acid (TAAA) of proteinaceous feedstuffs	70



## LIST OF FIGURES

FIGURE		PAGE
1	Average body weight through 8 weeks, Experiment 2a	41
2	Feed intake through 8 weeks, Experiment 2a	42
3	Feed/gain through 8 weeks, Experiment 2a	43
4	Average body weight through 8 weeks, Experiment 2b	56
5	Feed intake through 8 weeks, Experiment 2b	57
6	Feed/gain through 8 weeks, Experiment 2b	58



## LIST OF APPENDIX TABLES

TABLE		PAGE
1	Chemical analyses of starter diets, Experiment 2a	A 4
2	Chemical analyses of finisher diets, Experiment 2a	A 4
3	Chemical analyses of starter diets, Experiment 2b	A 5
4	Chemical analyses of finisher diets, Experiment 2b	A 5
5	Data on digestibility trial, Experiment 2a	A 6
6a	Data on total weight gain, performance trial of Experiment 2a	A 7
6b	Data on total intake, performance trial of Experiment 2a	A 8
6c	Data on feed/gain, performance trial of Experiment 2a	A 9
7	Data on digestibility trial, Experiment 2b	A10
8a	Data on total weight gain, performance trial of Experiment 2b	A11
8b	Data on total intake, performance trial of Experiment 2b	A12
8c	Data on feed/gain, performance trial of Experiment 2b	A13
9	Feed and faecal data for true metabolizable energy determination	A14
10	Amino acid composition of feed and faecal samples (% DM)	A15
11	Analysis of variance on digestibility of DM, Experiment 2a	A16
12	Analysis of variance on digestibility of CP, Experiment 2a	A16
13	Analysis of variance on digestibility of EE, Experiment 2a	A17
14	Analysis of variance on digestibility of CF, Experiment 2a	A17
15	Analysis of variance on digestibility of NFE, Experiment 2a	A18



TABLE		PAGE
16	Analysis of variance on total weight gain, Experiment 2a	A18
17	Analysis of variance on total intake, Experiment 2a	A19
18	Analysis of variance on feed/gain, Experiment 2a	A19
19	Analysis of variance on digestibility of DM, Experiment 2b	A20
20	Analysis of variance on digestibility of CP, Experiment 2b	A20
21	Analysis of variance on digestibility of EE, Experiment 2b	A21
22	Analysis of variance on digestibility of CF, Experiment 2b	A21
23	Analysis of variance on digestibility of NFE, Experiment 2b	A22
24	Analysis of variance on total weight gain, Experiment 2b	A22
25	Analysis of variance on total intake, Experiment 2b	A23
26	Analysis of variance on feed/gain, Experiment 2b	A23



## LIST OF ABBREVIATIONS

AAA	amino acid availability
AAAA	apparent amino acid availability
ADF	acid detergent fibre
ADL	acid detergent lignin
AME	apparent metabolizable energy
BE	bioavailable energy
CF	crude fibre
CP	crude protein
DCP	digestible crude protein
DM	dry matter
EE	ether extract
(FE + UE)	excreta energy
(FEn + UEn)	excreta energy corrected to zero nitrogen balance
FFM	Federal Flour Mill
(FN + UN)	faecal and urinary nitrogen
FSM	fish meal
(FmE + UeE)	Metabolic faecal plus endogenous urinary energy
(FmEn + UeEn)	nitrogen corrected energy excretion of unfed bird
g	gram
GE	gross energy
IE	energy input
IN	nitrogen input
kcal	kilocalorie
kg	kilogram
ME	metabolizable energy
MJ	megajoule
N	nitrogen
NDF	neutral detergent fibre
NE	net energy
NFE	nitrogen free extract
SBM	soybean meal
SS	Soon Soon
TAAA	true amino acid availability
TME	true metabolizable energy
TME <sub>n</sub>	TME corrected to zero nitrogen balance



## ABSTRACT

An abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia in partial fulfilment of the requirement for the Degree of Master of Science.

### NUTRITIVE VALUE AND BIOAVAILABILITY OF PROTEIN FEEDSTUFFS FOR BROILER CHICKENS

by

FAIRDA LIM

August, 1985

Supervisor: Professor Dr. Rudy I. Hutagalung

Faculty: Kedokteran Veterinar dan Sains Peternakan

Three experiments were conducted to evaluate the nutritive value and biological availability of nutrients in proteinaceous feedstuffs for broiler chickens. These include Thai fish meal (FSM), Peruvian FSM, China soybean meal (SBM), two locally-processed SBM, Federal Flour Mill (FFM) and Soon Soon (SS), respectively, and single-cell protein "Pruteen".

In Experiment 1, the nutritive values of these feedstuffs were subjected to the following chemical analyses: proximate, minerals, detergent fibre, amino acids and in vitro digestibility. Crude protein (CP) contents of fish meals (on dry matter basis) were



54.17% for Thai FSM and 60.98% for Peruvian FSM. Ash content in Thai FSM was higher (25.86%) than Peruvian FSM (15.51%). Gross energy in Peruvian FSM (4,488 kcal/g) was higher than Thai FSM (3,781 kcal/g), attributing mainly to higher fat content in the Peruvian FSM (10.37%) than in the Thai FSM (7.59%). In vitro evaluation of fish meals showed that the digestible CP (DCP) was higher for Thai FSM (42.2%) than the Peruvian FSM (40.0%). SBM of China origin showed highest protein content (44.41%) followed by FFM SBM (43.09%) and SS SBM (41.04%). However, the amino acid profiles showed that China SBM had the lowest total amino acid value (42.81%) and SS the highest (44.08%). In vitro evaluation showed that China SBM had the highest DCP (38.33%) and that of the two locally-processed SBM were comparable (35.15 and 35.64%). Pruteen had a high CP value (71.94%) and DCP (61.0%), but low in crude fibre (1%), and high in gross energy (4,527 kcal/g).

Experiment 2 consisted of two trials. In the first trial, the effects of Thai FSM (5 to 32%) and Pruteen (3 and 5%) inclusion in the diets on digestibility and performance by broiler chickens were studied. The results showed that diets containing FSM exhibited higher nutrient digestibility and superior performance than the control diet without added FSM. Optimal performance was obtained at 10 and 15% FSM inclusion. In the second trial the inclusion of Peruvian FSM (28/22%) was inferior to Thai FSM (32/25%) in nutrient digestibility and performance. Pruteen gave comparable nutrient digestibility and performance as those on FSM-based diets, although it was observed that there was growth depression and low feed intake at higher levels of Pruteen inclusion. The second trial studied the effect of type and level





of SBM-based diets on the digestibility and performance by broiler chickens. The data showed that digestibility and performance were superior at lower level (20%) of SBM inclusion in diets containing FSM than higher level (44/35%) of SBM with no added FSM. This study revealed that SS SBM had relatively lowest quality among the three SBM.

Experiment 3 was carried out to determine the true metabolizable energy (TME) as bioavailable energy, and amino acid availability of protein feedstuffs. The results showed that Thai FSM contained 3.30 kcal TME/g and Pruteen, 3.23 kcal TME/g. The TME for FFM SBM was the highest (2.92 kcal/g) and China, the lowest (2.69 kcal/g) of the SBM. Thai FSM and Pruteen showed high true amino acid availability (TAAA) (average 89.0 and 92.0%, respectively). The TAAA of China SBM was lowest (74.1%) whereas the two locally-processed SBM had the same TAAA (86.7 and 86.4%, respectively).



## CHAPTER I

### INTRODUCTION

Efficient animal production depends on the availability of feedstuffs of good quality and undoubtedly protein quality is an important measure. The quality of protein source is the most variable among feed ingredients and also generally, the more expensive in cost. Therefore, the study of feed protein quality deserves thorough assessment, involving the study of the general quality of feedstuffs, the amino acid composition and the bioavailability of nutrients to the animals.

Chemical analyses provide estimates of the nutritive quality of feedstuffs and play an important part in providing information on the amino acid profiles of feedstuffs. Bioassay, digestibility and performance trials need to be included to assess the nutritive value of feedstuffs adequately. Bioavailable nutrients such as amino acids and energy in feedstuffs are of fundamental importance in determining value. Knowledge of the amount and availability of various nutrients supplied by the feedstuffs is essential in order to avoid inefficient feeding and enormous wastage of feed resources.

The determination amino acid availability is time-consuming and expensive. This has delayed the publication of data on available amino acid requirements and the formulation of diets based on available amino acids. It is desirable to employ a simpler and more rapid method in determining the amino acid availabilities in feedstuffs.

True metabolizable energy (TME), an estimate of bioavailable energy, is important in estimating requirements and formulating rations. A rapid bioassay for TME in feedstuffs for poultry has been developed (Sibbald, 1976) and an adaptation of the TME procedure



(Likuski and Dorrell, 1978; Sibbald 1979b, c) can be used to assay for amino acid availability (AAA).

Optimal bird performance depends very much on diet formulation of correct amount of the various nutrient to meet accurately the quantitative nutritional requirements. Emphasis is placed on the relationships between compositional attributes and animal performance. Thus, the nutrient content and availability in feedstuffs is becoming important in livestock feeding and needs to be considered for precise diet formulation and preparation.

Feed formulation in Malaysia, at present relies on chemical composition and overseas nutrient availability data. It is not really appropriate to use these value, in view of the variability due to variety, location and methods of production of the locally available proteinaceous feedstuffs. However, work on nutrient availability of the locally available proteinaceous feedstuffs is still very much limited in Malaysia. Consequently, this study seeks to elucidate by chemical and biological evaluation, the quality, the TAAA and the TME of some of the locally available proteinaceous feedstuffs used in broiler diets. These include various fish meals and soybean meals. In addition, the recently introduced single cell protein in Malaysia, designated as Pruteen, produced from Methylophilus methylotrophus by anaerobic fermentation of methanol in the presence of ammonia was chosen for evaluation.



## CHAPTER II

### LITERATURE REVIEW

#### A. METHODS OF FEED EVALUATION

Evaluation of feedstuffs in the context of animal production is very important for efficient feeding to improve performance of the animals and to avoid enormous wastage of feed resources. The simplest evaluation of feedstuffs is a comparison of their gross effects as in feeding trials. A further refinement is the chemical analyses of feedstuffs and subsequently, determination of the digestible nutrients and bioavailabilities in the different feedstuffs. These chemical and biological means of evaluating feedstuffs are accepted standardised methods of providing information on composition and/or nutritive values.

##### 1. Chemical Evaluation

Chemistry is very important in predicting the value of feedstuffs. Chemical analyses provide means to determine the common nutrients such as protein and its amino acid profile, fibre, fat, ash, minerals and calorific content. These procedures are described in the book Official Methods of Analyses, published by the Association of Official Analytical Chemists (AOAC, 1975).

##### (i) Proximate Analysis

The need to develop and quantitative system



for classifying and evaluating feedstuffs has long been recognized by animal nutritionists. Henneberg and Stohman, working in the early part of the nineteenth century at the Weende Experimental Station in Germany, introduced the 'Weende' System of feed analysis which describes feeds in terms of their proximate components: moisture, ash, fat, fibre, crude protein and nitrogen-free extract. At present, the proximate analysis is probably the most generally used chemical scheme for describing feedstuffs.

The Weende analysis does not define the nutrient content of feedstuffs. It is an index of nutritive value only because the fractions that it isolates are correlated with some of the properties of feeds that have nutritional significance. Consequently, it is a useful descriptive device in establishing the characteristics of feedstuffs.

Although the Weende analysis is inadequate as a measure of the chemical composition, it provides useful information on the nutritive value of feedstuffs in general and is still retained as a routine method of analysis. In addition, most laboratories supplement the data obtained from proximate analysis with more detailed analyses carried out by modern techniques.



The general principles of proximate analysis are given below:-

- (a) Crude Protein (CP) - This is approximate by multiplying the Kjeldahl nitrogen value by the factor 6.25. Depending on the type of feedstuff, a greater or lesser part of the nitrogen is present as true protein while the remainder is inorganic nitrogen salts, amides, etc.
- (b) Ether Extract (Crude Fat)(EE) - This is measured as diethylether or petroleum ether extracted material. It includes not only oils and fats but fatty acids, resins, chlorophyll, etc.
- (c) Crude Fibre (CF) - This refers to organic matter insoluble in hot dilute sulphuric acid and dilute sodium hydroxide solution. It is noted that the CF in many cases is a misleading indication of the digestibility and nutritive value of a feedstuff as has been shown by the development of methods for partitioning the fibre fraction (Van Soest, 1967).
- (d) Ash - This is determined by igniting samples until they are free of carbon.
- (e) Nitrogen Free Extract (NFE) - This is the difference between the sum of the other



constituents and the original dry weight.

In other words, it is what remains after the other groups of components have been detected by analysis. NFE includes sugars, starches, etc.

(ii) Gross Energy

The animal obtains energy from its feeds. The energy present in the feed is measured by converting it into heat energy and determining the heat produced. The conversion is carried out by oxidizing the feed by oxidizing the feed by burning it; the quantity of heat resulting from the complete oxidation of unit weight of a feedstuff is known as the gross energy or heat of combustion of that feedstuff.

(iii) Minerals

It is important to know mineral contents of feedstuffs because of the great inherent variability. Macro-elements like calcium (Ca), phosphorus (P) and magnesium (Mg) may be measured. The ashed samples are digested in acids and analysed for the minerals. Ca and Mg are determined by atomic absorption, and P is determined by colorimetric method, involving the vanadomolybdate complex.

(iv) Fibre Analysis

Crude fibre has been and remains a common means of evaluating fibre content in feedstuffs.

However, the need for better methods of the fibre analysis arises due to the deficiencies of crude fibre analysis and associated proximate analysis (Van Soest, 1977). The weaknesses of the CF method have been known for a long time but have only recently become widely recognized. The error of the CF assay stems from its failure to recover lignin and other fibre components (Van Soest, 1975).

Fibres are variable in their composition and properties, and it is not possible to describe the characteristics and amount of fibre with a single value. They will require analysis for components including lignin, cellulose and hemicellulose.

#### The Detergent System of Fibre Analyses

The objective of the detergent system of analyses is to fractionate feeds of plant origin relative to their nutritive availability and fibre content. The cell wall components of feed are recovered in the neutral detergent fibre (NDF), while the acid detergent fibre (ADF) divides these into fractions soluble and insoluble in IN acid. The acid soluble fraction includes the hemicellulose and cell wall protein while the residue (ADF) recovers cellulose and lignin which is the least digestible non-carbohydrate fractions. Improved systems for fibre analysis (Goering and Van Soest, 1970) have been widely accepted in research laboratories as replacements





for the older crude fibre technique.

Studies have been conducted in the use of the detergent fibre analyses in formulating broiler rations (Strong and Holder, 1978). The study concluded that ADF is a more accurate and repeatable analysis for fibre than is CF. Thus, when data are available, ADF might be preferable to CF in formulating poultry diets.

(v) Amino Acid

The crude protein value does not distinguish between protein and non-protein nitrogen and assumes that all protein contains 16% nitrogen and is of equal biological availability. It gives no indication of the content of amino acids which constitute the protein and which are required by the animal. The amino acid composition and levels are useful in predicting the potential nutritive value of the protein to the animal.

The amino acid composition and levels can be determined accurately by ion-exchange chromatography of protein hydrolysates.

(iv) In Vitro Evaluation

In vitro evaluation provides a means of rapid assessment of nutrient bioavailability. Procedures have been developed and modified to imitate the action of the animal digestive system and are used