NUTRITIONAL EVALUATION OF SOME BANGLADESHI OILSEED BY-PRODUCTS AS DIETARY PROTEIN SOURCES FOR COMMON CARP (*Cyprinus carpio* L)

Thesis submitted to the University of Stirling
for the degree of Doctor of Philosophy

By

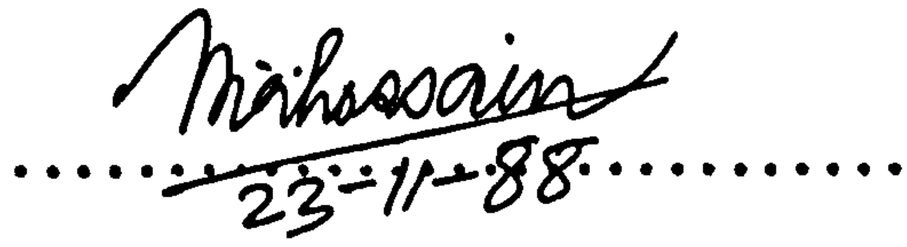
MD. ARSHAD HOSSAIN
BSc Fisheries (Hons), MSc

Institute of Aquaculture
University of Stirling
Stirling
Scotland

November 1988

DECLARATION

I hereby declare that this thesis has been composed by myself and is the result of my own investigations. It has neither been accepted, nor is being submitted for any other degree. All sources of information have been duly acknowledged.



23-11-88

To my -

Beloved Parents

LIST OF CONTENTS

		Page
List of Tables		(i)
List of Figures		(vii)
List of Plates		(viii)
Acknowledgements		(ix)
Abstract		(x)
CHAPTER 1 : GENERAL INTRODUCTION		
1.1	Aquaculture in Asia	1
1.2	Aquaculture in Bangladesh	4
1.2.1	Background	4
1.2.2	Carp culture in Bangladesh and present constraints to development	9
1.3.1	Requirement for feeds and feedstuffs for aquaculture in Bangladesh	13
1.3.2	Availability of feed and feedstuffs in Bangladesh	15
1.3.3	Selection of various oilseed cakes as alternative protein sources to fish meal	18
1.4	Important anti-nutritional factors in the selected feed ingredients	19
1.4.1	Glucosinolates	19
1.4.2	Phytic acid	23
1.4.3	Cyanogenetic glucosides	27
1.4.4	Tannins	29
1.5	The Aims of the Research	29
CHAPTER 2 : GENERAL MATERIALS AND METHODS		
2.1	Experimental facilities	31
2.1.1	System for growth trials	31
2.1.2	Faecal collection system	36
2.2.2	Quarantine procedures	42
2.2.3	Acclimation and weighing procedures	44
2.2.4	Diet formulation	44
2.2.5	Diet preparation	45
2.3.1	Analysis of proximate composition	49
2.3.2	Chromic oxide analysis	50
2.4.1	Amino acid analysis	50
2.4.2	Available lysine analysis	51

		Page
2.5.1	Energy determination	52
2.6.1	Mineral analysis	52
2.7.1	Anti-nutritional factors analysis	53
2.8.1	Analysis of experimental data	53
2.9.1	Statistical analysis	57

CHAPTER 3 : EXPERIMENT 1
Substitution of fish meal by locally available Bangladeshi oilseed by-products in the diet of common carp (Cyprinus carpio L)

3.1	Introduction	58
3.2	Materials and Methods	61
3.2.1	Experimental system	61
3.2.2	Analytical techniques	61
3.2.3	Diet formulation and preparation	62
3.2.4	General experimental procedure	71
3.2.5	Feeding rates	71
3.2.5	Faeces collection	72
3.2.7	Histological techniques	72
3.2.8	Analysis of experimental data	73
3.3	Results	73
3.3.1	Analysed composition of the experimental diets	73
3.3.2	Acceptability of the diets	74
3.3.3	Mortality	74
3.3.4	General health and histological examination	74
3.3.5	Growth	82
3.3.6	Food conversion ratio	85
3.3.7	Protein utilization	85
3.3.8	Apparent nutrient digestibility	86
3.3.9	Carcass composition	89
3.4	Discussion	89

CHAPTER 4 : EXPERIMENT 2
Effect of dietary essential amino acid supplementation on growth and feed utilization in common carp (C. carpio L)

4.1	Introduction	100
4.2	Materials and Methods	102
4.2.1	Experimental system and animals	102
4.2.2	Analytical techniques	102
4.2.3	Diet formulation and preparation	103
4.2.5	Feeding rates	109
4.2.6	Faeces collection	110
4.2.7	Histological techniques	110
4.2.8	Analysis of the experimental data	110

Page

4.3 Results 110

4.3.1 Analysed composition of the diets 111
4.3.2 Growth 111
4.3.3 Food conversion ratio 114
4.3.4 Protein utilization 115
4.3.5 Apparent nutrient digestibility 115
4.3.6 Carcass composition 118
4.3.7 General health and histological examination 119

4.4 Discussion 119

CHAPTER 5 : EXPERIMENT 3
Studies on the protein, energy and amino acid digestibility of fish meal, mustard oilcake, linseed and sesame meal for common carp (C. carpio L)

5.1 Introduction 127

5.2 Materials and Methods 131

5.2.1 Experimental system and animals 131
5.2.2 Analytical techniques 131
5.2.3 Diet formulation and preparation 132
5.2.4 General experimental procedure 136
5.2.5 Feeding rates 136
5.2.6 Faeces collection 136
5.2.7 Analysis of experimental data 138
5.2.8 Statistical analysis 138

5.3 Results 139

5.3.1 Analysed composition of the diets 139
5.3.2 Faecal amino acids composition 139
5.3.3 Protein and energy digestibility 144
5.3.4 Amino acids digestibility 144

5.4 Discussion 147

CHAPTER 6 : EXPERIMENT 4.1
Detoxification of mustard oilcake and evaluation of its nutritive value in the diet of common carp (C. carpio L)

6.1 Introduction 155

6.2 Materials and Methods 159

6.2.1 Experimental system and animals 159
6.2.2 Analytical techniques 159
6.2.3 Detoxification of mustard oilcake 160
6.2.4 Diet formulation and preparation 164
6.2.5 General experimental procedure 169
6.2.6 Feeding rates 169

		Page
6.2.7	Faeces collection	169
6.2.8	Histological techniques	170
6.2.9	Analysis of the experimental data	170
6.3	Results	170
6.3.1	Effect of detoxification on the chemical composition of mustard oilcake	170
6.3.2	Growth	171
6.3.3	Food conversion ratio	174
6.3.4	Protein utilization	174
6.3.5	Apparent nutrient digestibility	175
6.3.6	Carcass composition	175
6.3.7	General health and histological examination	178
6.4	Discussion	178
CHAPTER 6 : EXPERIMENT 4.2	**Detoxification of linseed and sesame meal and evaluation of their nutritive value in the diets of common carp (C. carpio L)**	
6.5	Introduction	185
6.6	Materials and Methods	188
6.6.1	Experimental systems and animals	188
6.6.2	Analytical techniques	189
6.6.3	Detoxification of linseed and sesame meals	189
6.6.4	Diet formulation and preparation	189
6.6.5	General experimental procedure	193
6.6.6	Feeding rates	198
6.6.7	Faeces collection	198
6.6.8	Histological techniques	198
6.6.9	Analysis of experimental data	198
6.7	Results	199
6.7.1	Effect of detoxification on the chemical composition of linseed and sesame meals	199
6.7.2	Growth	199
6.7.3	Food conversion ratio	202
6.7.4	Protein utilization	202
6.7.5	Apparent nutrient digestibility	203
6.7.6	Carcass composition	203
6.7.7	General health and histological examination	206
6.8	Discussion	206

Page

CHAPTER 7 : EXPERIMENT 5.1
Effect of phytic acid in purified diets containing varying levels of calcium and magnesium on growth and mineral availability in common carp (C. carpio L)

7.1 Introduction 213

7.2 Materials and Methods 216

7.2.1 Experimental systems and animals 216
7.2.2 Analytical techniques 216
7.2.3 Diet formulation and preparation 217
7.2.4 General experimental procedures 224
7.2.5 Feeding rates 224
7.2.6 Faeces collection 224
7.2.7 Histological techniques 224
7.2.8 Analysis of experimental data 225

7.3 Results 225

7.3.1 Analysed composition of the diets 225
7.3.2 Growth 225
7.3.3 Food conversion ratio (FCR) 226
7.3.4 Protein utilization 229
7.3.5 Plasma mineral concentrations 229
7.3.6 Liver mineral contents 233
7.3.7 Kidney mineral contents 233
7.3.8 Carcass composition 234
7.3.9 Carcass mineral contents 234
7.3.10 General health and histological examination 234

7.4 Discussion 239

CHAPTER 8 : EXPERIMENT 6
Partial substitution of fish meal by oilseed meals in various combination in the diet of common carp (C. carpio L)

8.1 Introduction 273

8.2 Materials and Methods 275

8.2.1 Experimental system and animals 275
8.2.2 Analytical techniques 275
8.2.3 Diet formulation and preparation 275
8.2.4 General experimental procedure 282
8.2.5 Feeding rates 282
8.2.6 Faeces collection 283
8.2.7 Histological techniques 283
8.2.8 Analysis of the experimental data 283

8.3 Results 283

8.3.1 Analysed composition of the diets 283

		Page
8.3.2	Growth	284
8.3.3	Food conversion ratio	287
8.3.4	Protein utilization	287
8.3.5	Nutrient digestibility	288
8.3.6	Carcass composition	288
8.3.7	General health and histological examination	291
8.4	Discussion	291

CHAPTER 9 : GENERAL DISCUSSION AND CONCLUSION

9.1	General Discussion	296
9.2	Suggestion for Future Work	304
9.3	Conclusions	305

REFERENCES 309

LIST OF TABLES

Table No.		Page
1.1	Aquaculture production in Asia in 1983 (in tonnes)	3
1.2	Estimated inland water resources of Bangladesh with fish production for the year 1983-84	6
1.3	Species which are cultured at present and of likely future culture potential in Bangladesh	7
1.4	The benchmark production of fish in 1984/85 and the target for 1989/90 by various types of water bodies	8
1.5	Summary of feed, fertilizer and chemical needs for 2005	14
1.6	Oil crops in Bangladesh	17
1.7	Different types and levels of glucosinolate in mustard seed	22
1.8	Typical phytic acid contents of some cereals and oilseeds	24
2.1	Various sources of fry supply used in different experiments	43
2.2	A summary of nutrient requirement of carp fry and fingerlings	46
2.3	Composition of mineral premixes used in experimental diets	47
2.4	Composition of the vitamin premixes used in experimental diets	48
3.1	Proximate composition, Ca, P, anti-nutritional factors and available lysine content of the dietary ingredients used in Experiments 1 and 2 (% dry matter basis)	64
3.2	Essential amino acid (EAA) composition (% dry matter) of the dietary protein sources used in Experiments 1 and 2	65
3.3	Level of inclusion of different test proteins (as % total crude protein) in the diets used in Experiment 1	66
3.4	Formulations of the experimental diets used in Experiment 1	67
3.5	Analysed composition of the experimental diets used in Experiment 1 (% dry matter basis unless otherwise stated)	68

		Page
3.6	Analysed essential amino acid (EAA) composition of the diets used in Experiment 1 (% dry matter basis)	69
3.7	A summary of the methodology used to evaluate the plant protein sources locally available in Bangladesh as substitute for fish meal in carp fry diet	70
3.8	Observation on the acceptability of different diets containing plant proteins fed to carp fry	75
3.9	Growth and food utilization of common carp fry fed the experimental diets for 8 weeks	83
3.10	Apparent nutrient digestibility (%) of the experimental diets	87
3.11	Proximate carcass composition analysis (% fresh matter basis) of fish samples at the start and the end of the experiment	88
4.1	Level of inclusion of different test protein (as % of total protein) and the amino acids supplemented in the diets used in Experiment 2	104
4.2	Composition (%) of the experimental diets used in Experiment 2	105
4.3	Analysed proximate composition of the experimental diets used in Experiment 2 (% dry matter basis unless otherwise stated)	106
4.4	Analysed essential amino acid (EAA) composition of the experimental diets used in Experiment 2 (% dry matter basis)	107
4.5	A summary of the methodology used to study the effect of supplementation of limiting dietary essential amino acid (EAA) on growth and feed utilization in carp	108
4.6	Growth and food utilization of common carp fry fed the experimental diets for 8 weeks	112
4.7	Apparent nutrient digestibility of experimental diets	116
4.8	Proximate carcass composition analysis (% fresh weight basis) of fish samples at the start and at the end of the experiment	117
5.1	Proximate composition of the dietary ingredients used in Experiment 3 (% dry matter basis)	133

		Page
5.2	Amino acid composition (% dry matter) of the dietary protein sources used in Experiment 3	134
5.3	Composition (%) of the experimental diets used in Experiment 3	135
5.4	A summary of the methodology used to study the digestibility of experimental diets	137
5.5	Analysed proximate composition of the experimental diets used in Experiment 3 (% dry matter basis unless otherwise stated)	140
5.6	Analysed amino acid composition of the experimental diets used in Experiment 3 (% dry matter basis)	141
5.7	Faecal amino acid pattern of carp fed the experimental diets (% dry matter basis)	142
5.8	Faecal amino acid pattern of carp fed a protein-free diet as compared to those of channel catfish and rainbow trout	143
5.9	Apparent protein, energy and amino acid digestibility (%) of the experimental diets	145
5.10	True protein and amino acid digestibility (%) of the experimental diets	146
5.11	Protein digestion coefficient of some commonly used feed ingredients for carp, channel catfish and rainbow trout	148
6.1	Proximate composition of dietary ingredients used in Experiment 4.1 (% dry matter basis)	161
6.2	Amino acid composition (% dry matter) of the dietary protein sources used in Experiment 4.1	162
6.3	Effect of different processing treatments on available lysine, allyl isothiocyanate and tannin contents (% dry matter basis) of mustard oilcake	163
6.4	Composition of the experimental diets used in Experiment 4.1	165
6.5	Analysed proximate composition of the experimental diets used in Experiment 4.1 (% dry matter basis unless otherwise stated)	166

		Page
6.6	Amino acid composition of the experimental diets used in Experiment 4.1 (% dry matter basis)	167
6.7	A summary of the methodology used to study the nutritive value of detoxified MOC on the growth and food utilization in carp	168
6.8	Growth and food utilization of common carp fed the experimental diets for 8 weeks	172
6.9	Apparent nutrient digestibility (%) of the experimental diets	176
6.10	Proximate carcass composition (% fresh matter basis) of fish samples at the start and the end of the experiment	177
6.11	Proximate composition of dietary ingredients used in Experiment 4.2 (% dry matter basis)	190
6.12	Amino acid composition (% dry matter) of the dietary protein sources used in Experiment 4.2	191
6.13	Effect of different processing treatments on available lysine, phytic acid and hydrocyanic acid contents (% dry matter basis) of linseed and sesame meal	192
6.14	Composition of experimental diets used in Experiment 4.2	194
6.15	Analysed proximate composition of the experimental diets used in Experiment 4.2 (% dry matter basis unless otherwise stated)	195
6.16	Amino acid composition of the experimental diets used in Experiment 4.2 (% dry matter basis)	196
6.17	A summary of the methodology used to study the nutritive value of detoxified linseed and sesame meals on the growth and food utilization in carp	197
6.18	Growth and food utilization of common carp fed the experimental diets for 8 weeks	200
6.19	Apparent nutrient digestibility of the experimental diets	204
6.20	Proximate carcass composition (% fresh matter basis) of fish samples at the start and the end of the experiment	205
7.1	Specification of the experimental diets used to study the effect of phytic acid on the growth of carp	218
7.2	Formulation of experimental diets used in Experiment 5.1	219

		Page
7.3	Analysed composition of the experimental diets used in Experiment 5.1 (% dry matter basis unless otherwise stated)	220
7.4	Mineral composition of the test diets used in Experiment 5.1 (dry matter basis)	221
7.5	Amino acid composition of the test diets used in Experiment 5.1 (% dry matter basis)	222
7.6	A summary of the methodology used to study the effect of phytic acid in purified diets containing varying levels of calcium and magnesium on the growth of common carp	223
7.7	Growth and food utilization of common carp fed the experimental diets for 8 weeks	227
7.8	Final mean plasma mineral concentration (mg/l) in carp fed the experimental diets	230
7.9	Final mean liver mineral content (mg/kg, fresh matter basis) of carp fed the experimental diets	231
7.10	Final mean kidney mineral content (mg/kg, fresh matter basis) of carp fed the experimental diets	232
7.11	Proximate carcass composition analysis (% fresh weight basis) of fish at the start and the end of the experiment	235
7.12	Final mean carcass mineral composition (fresh matter basis) of carp fed experimental diets	236
7.13	Specification of the experimental diets	250
7.14	Formulations of the diets used in Experiment 5.2	251
7.15	Analysed composition of the diets used in Experiment 5.2 (% dry matter basis)	252
7.16	Analysed amino acid composition of the diets used in Experiment 5.2 (% dry matter basis)	253
7.17	A summary of the methodology used to evaluate the effects of glucosinolate (allyl isothiocyanate) on growth and food utilization of common carp	254
7.18	Growth and food utilization of common carp fed the experimental diets for 8 weeks	258

		Page
7.19	Apparent nutrient digestibility (%) of the experimental diets	262
7.20	Proximate carcass composition (% fresh matter basis) of fish samples at the start and the end of the experiment	263
8.1	Specification of the experimental diets used in Experiment 6	276
8.2	Proximate composition and anti-nutritional factors contents of the ingredients used in Experiment 6 (% dry matter basis)	277
8.3	Formulations of the diets used in Experiment 6	278
8.4	Analysed composition of the diets used in Experiment 6 (% dry matter basis)	279
8.5	Analysed amino acid composition of the diets used in Experiment 6 (% dry matter basis)	280
8.6	A summary of the methodology used to evaluate the oilseed meals as substitute for fish meal in the diet of common carp	281
8.7	Growth and food utilization of common carp fed the experimental diets for 8 weeks	285
8.8	Apparent dry matter, protein, lipid and amino acid digestibilities (%) for the experimental diets	289
8.9	Proximate carcass composition (% fresh matter basis) of fish samples at the start and the end of the experiment	290

LIST OF FIGURES

Figure No		Page
1.1	The enzymatic hydrolysis of glucosinolate	21
1.2	The enzymatic hydrolysis of allyl thioglucoside (sinigrin) to allyl isothiocyanate	21
1.3	Structure of phytic acid (Proposed by Anderson, 1914)	25
1.4	Structure of phytic acid (Suggested by Neuberg, 1908)	26
1.5	The enzymatic hydrolysis of linamarin, the cyanogenetic glucoside of linseed	28
2.1.a	Diagrammatic representation of the recirculated water system used for the growth trial in all the experiments	33
2.1.b	Diagrammatic representation of one of the experimental tanks in recirculated water system used in growth trials	35
2.2.a	Diagrammatic representation of the faecal collection system	39
2.2.b	Diagrammatic representation of one of the tanks used in the faecal collection	41
3.1	Growth responses of fish fed experimental diets for 8 weeks	84
4.1	Growth responses of fish fed experimental diets for 8 weeks	113
6.1	Growth responses of fish fed experimental diets for 8 weeks	173
6.2	Growth responses of fish fed experimental diets for 8 weeks	201
7.1	Growth responses of fish fed experimental diets for 8 weeks	228
7.2	Growth responses of fish fed experimental diets for 8 weeks	259
8.1	Growth responses of fish fed experimental diets for 8 weeks	286

LIST OF PLATES

Plate No.		Page
3.1	Lateral view of carp fed Diet 8 (75% sesame meal protein) from Experiment 1 showing poor health	76
3.2	Fish fed the fish meal based Control diet from Experiment 1 showing normal heatlh	76
3.3	Thyroid follicles in kidney tissues of carp fed fish meal based Control diet from Experiment 1	78
3.4	Thyroid follicles in kidney tissues of carp fed Diet 3 (50% mustard protein) from Experiment 1 showing variable sizes, shapes and general packed appearance of the follicles	78
3.5	Section of liver from carp fed Diet 3 (50% mustard protein) from Experiment 1 showing severe intra-cellular lipid deposition	80
3.6	Section of liver from carp fed fish meal based Control diet from Experiment 1 showing no visible intra-cellular fat deposition	80
7.1.a	Section of intestine of carp fed diet containing high phytic acid, calcium and magnesium content (Diet 9) from Experiment 5.1 showing marked hypertrophy and marked vacuolization of the cytoplasm	238
7.1.b	Section of intestine of carp fed casein and gelatin based Control diet from Experiment 5.1 showing normal columnar shaped cells with fine granular cytoplasm	238
7.2	Thyroid follicles in kidney tissues of carp fed diets (4, 5, 8 and 9) containing higher levels of allyl isothiocyanate from Experiment 5.2 showing variable sizes and general packed appearance of follicles	265
7.3.a	Thyroid follicles in pharyngeal region of carp fed diets (4, 5, 8 and 9) containing higher levels of allyl isothiocyanate from Experiment 5.2 showing marked epithelial hyperplasia	267
7.3.b	Thyroid follicles in pharyngeal region of carp fed fish meal based Control diet from Experiment 5.2 showing no visible epithelial hyperplasia	267

ACKNOWLEDGEMENTS

I would like to thank Dr Kim Jauncey for his advice, encouragement and supervision of the research project and for seeing this thesis through to its conclusion. My gratitude to Professor R. J. Roberts for his encouragement throughout my stay in Stirling.

Special thanks are due to Dr. D. J. Macintosh and the Office of the British High Commission, Dhaka, Bangladesh, for making the necessary arrangements for sending the feed ingredients from Bangladesh for this research project.

Thanks are also due to Dr. M. R. Hasan and Dr. M. A. Wahab for advice and encouragement during their stay in Stirling. I would like to thank Mrs. Betty Stenhouse, Mr. Alan Porter, Mr. Paul Thomson and Mr. Billy Struther and other technical staff of the Institute of Aquaculture for their kind help throughout the experimental work.

Sincere appreciation to my friends and all the PhD students for their help and many lively discussions. A special thanks to Joanna Mackay for her patience and excellent typing of the manuscript.

Finally I extend my most sincere thanks to my wife Shahnaz for love, sacrifice and practical help throughout my PhD study.

ABSTRACT

The nutritional suitability of some Bangladeshi oilseed by-products (mustard, Brassica juncea; linseed, Linum usitatissimum; sesame, Sesamum indicum) as fish meal substitutes in carp diets was investigated.

These protein sources were shown to cause depressed growth and feed efficiency when substituting 25% or more of the fish meal protein in semi-purified diets. However, the use of these oilseed meals in combination was found to be more effective than that of single sources.

Supplementation of plant protein diets with crystalline EAA improved their nutritive value. Growth performance was better in fish fed diets supplemented with all deficient EAA than in fish fed diets supplemented with the first limiting EAA.

Nutrient digestibility studies with these plant proteins suggested reasonable agreement between apparent protein digestibility (APD) and average apparent amino acid digestibility (AAAD). APD and AAAD values ranged from 78.9% to 85% and 82.4% to 85.8% respectively.

Both aqueous and enzyme treatments were effective in reducing (49% and 57% respectively) the anti-nutritional factors (e.g. allyl isothiocyanate) in mustard oilcake. In linseed and sesame meals heat treatment was the most effective (reducing phytic acid levels by 72% and 74% respectively). Use of detoxified meals in diets improved growth performance and food utilization compared to untreated meals.

Dietary phytic acid in the presence of increased levels of calcium and magnesium significantly ($p < 0.05$) depressed growth, food utilization and mineral bioavailability (especially Ca and Zn) in carp.

Carp were shown to be tolerant of a dietary glucosinolate (allyl isothiocyante) level of 0.4 mg glucosinolate/g diet without inhibiting growth performance or adverse effects on fish health. However, fish fed diets containing higher levels of mustard oilcake or allyl isothiocyanate showed abnormal changes in thyroid tissues.

The results of this study are discussed in relation to previously published research on fish and other monogastric animals.

CHAPTER 1 : GENERAL INTRODUCTION

1.1 AQUACULTURE IN ASIA

Although aquaculture in Asia has a history of at least 3500 years the modern industry is still in its infancy in many Asian countries (Chua, 1986b). The long gestation period in aquaculture development is in part due to lack of economic pressure and in part to insufficient technical and financial inputs to demonstrate its commercial viability.

Fishing and aquaculture industries play a significant role in supplying fish protein to a large Asian population, many of whom suffer from chronic malnutrition (Ravenholt, 1982). Fish contributes a relatively large proportion of animal protein intake and accounts for 33% of the, relatively little, animal protein consumed by average Asian people (RAPA, 1985). In Southeast Asia, this share may be higher than 50% as the amount of meat protein consumed is relatively low (Florentino et al., 1985).

Although per capita fish consumption in East and Southeast Asian countries like Japan (83 kg), Hongkong (37 kg), Philippines (41 kg), Malaysia (43 kg) and Taiwan (35 kg), are the highest in the world, the South Asian countries consume relatively less fish than their neighbours. For example, per capita consumption in Nepal is less than 1 kg and it is only 7.3 kg in Bangladesh (Chua, 1986b).

Asia contributes the largest proportion, about 70% of the world fish production through aquaculture. A large proportion of farmed finfish production is from freshwater aquaculture. More than 1.7×10^6 tonnes

of freshwater fish were harvested in 1983 by China, Bangladesh, India, Burma, Indonesia, Nepal, Pakistan, Sri Lanka, Japan and Thailand. Most of the production was of Chinese and Indian carps (Chua, 1986a).

Many South Asian nations have no tradition of aquaculture but most have recently developed ambitious programmes to increase fish production. Compared to East and Southeast Asian countries, their contributions are relatively low (Table 1.1). Total farmed fish production in these countries, including Bangladesh, India, Nepal, Pakistan and Sri Lanka was about 300,000 tonnes in 1983 (Chua, 1986a) contributing only 6% of total Asian production. Although Bangladesh and India have a long history of fish farming, total production is still low considering the potential of their vast aquatic resources.

Some of the major constraints in encouraging private investment in aquaculture, especially in countries where there are no traditional aquaculture practices, are a lack of qualified, experienced personnel, a lack of relevant technical and economic information on pilot farms and poor supply and distribution services such as seeds, feeds and fertilizers (Pillay, 1981).

There is gradual progress from extensive to semi-intensive and then to intensive fish farming using artificial feeds and employing scientific management skills to increase yield in many developing countries which reflects the increasing application of scientific research results in aquaculture. In spite of recent advances in aquaculture, there are certain problems that require further scientific investigation. The

TABLE 1.1

Aquaculture production in Asia in 1983 (in tonnes)[1]

Country	Finfish	Mollusc	Crustacean	Seaweed	TOTAL	% of total fish production
Bangladesh	114,090	-	-	-	114,090	15.2
Burma	3,946	-	-	-	3,946	0.6
India	131,000	-	10,000	-	141,000	5.4
Nepal	4,000	-	-	-	4,000	64.5
Pakistan	5,004	-	-	-	5,004	1.5
Sri Lanka	35,530	-	-	-	35,530	16.2
Malaysia	16,820	49,462	245	-	66,527	9.0
Philippines	277,710	29,808	910	132,204	440,632	22.7
Indonesia	226,000	-	30,000	6,000	262,000	12.0
Singapore	861	979	179	-	2,014	10.3
Thailand	47,082	11,582	11,474	-	174,138	7.7
China	1,465,639	257,123	8,975	241,533	1,973,270	36.2
Taiwan	151,757	70,653	10,632	9,716	242,758	26.0
Hong Kong	8,060	60	-	-	8,120	4.3
South Korea	1,218	289,704	50	347,227	638,199	21.5
Japan	269,834	332,000	10,000	521,000	1,132,834	10.9

[1]Data from Chua (1986a)

industry still lacks easily available, cheap but effective, formulated feeds for finfish and especially for shrimp. High price artificial feeds may increase production costs and reduce profitability.

Therefore, adaptive research should be conducted in the production of artificial feeds using local ingredients and increasing the use of plant proteins instead of a total reliance on animal protein.

1.2 AQUACULTURE IN BANGLADESH

1.2.1 Background

Fishery products contribute an average of 20% of the animal proteins in the human diet (Barnes and Mann, 1980). In most areas of the tropics fish protein is even more important. Fish is an important item in the diet of the people of Bangladesh and supplies more than 80% of the animal protein (Haque, 1985). The fisheries sector contributes about 6% of the country's GNP. Fisheries as a source of employment, ranks second to Agriculture with about 6% of the population dependent directly or indirectly on fisheries for their livelihood (Ali, 1985). In 1984-85, exports of fisheries products contributed about 9.6% of total export earnings (Aquatic Farms, 1986).

Bangladesh has excellent aquaculture potential because of its vast water resources; yet per capita consumption has shown a severe decline over the last two decades. In 1962/63 the average consumption of fish was 12kg/yr per capita: it declined to 7.3 kg in 1979/80 and showed a

nominal increase to 7.6 kg in 1984/85 (TFYP, 1985-90). During 1980-85, fish availaibility at home (net of export) increased at an annual rate of 3.6% against a population growth of 2.4%. This small increase over the population growth rate was not enough to stop deterioration in fish intake (TFYP, 1985-90).

Bangladesh has vast water resources in the form of rivers, canals, depression (haors and beels) reservoirs, ox-bow lakes (baors), ponds, tanks, seasonal flooded areas and the Bay of Bengal - which has a 480 km long coastline and approximately 1 million hectares of territorial waters. The Bangladesh Fisheries Resources Survey System (BFRSS, 1984) estimated the inland water resources and the total fish production for the year 1983-84 which are shown in Table 1.2. These waterbodies have considerable potential for the production of finfish and shellfish. The inland waters are inhabited by about 257 native and six exotic species of fish and 13 species of prawns. About 475 species of fish and 25 species of shrimp are present in the marine waters of Bangladesh. Of 475 species of fish, 65 species are of commercial importance (Ali, 1985). Other aquatic animals include several species of crabs and turtles, two species of pearl producing mussels (Lamellidens sp and Parreysia sp) and one species of frog (Rana tigrina) (Karim, 1978).

Among the large number of indigenous species not all are suitable for aquaculture. Table 1.3 lists the species currently being cultured and of likely future significance in Bangladesh (Islam, 1983).

In Bangladesh, fish culture in ponds has been practised on a limited

TABLE 1.2

Estimated inland water resources in Bangladesh with fish production for the year 1983-84

		Area (Hectares)	Total catch (Tonnes)
A.	INLAND FISHERIES		
(a)	Capture Fisheries		
1.	Rivers including estuaries	1,031,563	207,000
2.	Beels and haors*	114,793	51,660
3.	Kaptai Lake	68,800	4,057
4.	Seasonal flood lands	2,832,792	202,000
	Sub Total	4,047,948	464,717
(b)	Culture Fisheries		
1.	Ponds	163,492	105,000
2.	Baors*	5,488	862
3.	Coastal aquaculture	51,812	8,228
	Sub total	220,792	114,090
	TOTAL	4,268,740	578,807
B.	MARINE FISHERIES		172,486
	GROSS TOTAL		751,293

Source: Bangladesh Fisheries Resources Survey System (BFRSS), 1984

* Beel: Small low-lying depression on a floodplain which may or may not dry up in the dry season

Haor: Large low-lying depression on a floodplain, part of which dries up after the rainy season ends. An inland sea. As the water level drops, individual beels may be formed

Baor: Oxbow lake. Closed water body, isolated from the river by a change in its course

TABLE 1.3

Species which are cultured at present and of likely future culture potential in Bangladesh

SCIENTIFIC NAME	LOCAL NAME	ENGLISH NAME
Catla catla	Catla	Catla
Labeo rohita	Rui	Ruhu
Labeo calbasu	Kalibaush	Calbasu
Cirrhina mrigala	Mrigal	Mrigal
Cyrpinus carpio	Carpo	Common carp
Hypophthalmichthys molitrix	Silver carp	Silver carp
Ctenopharyngodon idella	Grass carp	Grass carp
Clarias batrachus	Magur	Walking Catfish
Macrobrachium rosenbergii	Golda chingri	Giant fresh water prawn
Penaeus monodon	Bagda chingri	Giant tiger prawn
Species of likely future culture potential		
Cirrhina reba	Vanga	Reba
Puntius sarana	Sharputi	Puntius carp
Channa striatus	Shoal	Snakehead (Murrel)
Channa punctatus	Taki	"
Channa marulius	Gazar	"
Anabas testudineus	Koi	Climbing perch
Heteropneustes fossilis	Shingi	Catfish
Pangasius pangasius	Pangus	Catfish
Wallago attu	Boal	Catfish
Notopterus chitala	Chital	Featherback

Source: (Islam, 1983)

TABLE 1.4

The benchmark production of fish in 1984/85 and the target for 1989/90 by various types of water bodies

WATER BODIES		1984/85	1989/90
		(In '000' tonnes)	
I.	INLAND FISHERIES	558.4	772.4
(i)	Ponds	112.5	192.0
(ii)	Beels	1.1	2.8
(iii)	Coastal aquaculture	9.0	34.0
(iv)	River and Estuaries	207.1	250.4
(v)	Beels and Haors	51.7	75.0
(vi)	Kaptai Lake	5.0	8.0
(vii)	Flood plain	202.0	210.0
II.	MARINE FISHERIES	185.6	228.0
	TOTAL	774.0	1000.4

Source: Third Five Year Plan (1985-90) for Bangladesh

scale for some time. In the past the abundance of wild fish from natural inland fisheries considerably reduced interest in fish culture. During recent decades, however, overfishing and construction of flood and tide control dykes without protecting the interests of fisheries have depleted the natural stock substantially (Islam, 1983). As a result, per capita consumption of fish has declined. This has led to renewed interest in the potential of fish culture in both private and public sectors. The government of Bangladesh has placed great emphasis on fish production by intensification of aquaculture together with scientific management of open water fisheries in its Third Five Year Plan (1985-90). The government has set its production target at 1 million tonnes by 1989/90 from the present 0.77 million tonnes in 1984/85, as shown in Table 1.4.

1.2.2 Carp culture in Bangladesh and present constraints to development

Bangladeshi aquaculture has two dominant facets - inland carp culture and brackish water shrimp culture in coastal areas. Both culture systems are essentially extensive in nature. Each farming type is presently in the early stages of intensification with shrimp culture more advanced than carp farming (FAO/UNDP, 1985).

Bangladesh has a history of carp culture which dates back hundreds of years (Aquatic Farms, 1986). Traditionally farmers used their ponds to trap wild fish from annual floods. However, the culture of 'major' carp in ponds on a limited scale has been practised for a long time (Islam, 1983). Current fish culture practices in Bangladesh are simple.

Farmers stock fingerlings and wait until the fish are large enough to harvest. Actual stocking is still not practised in all ponds nor is enhancement of production through fertilizer or feed widely used. As a result, there is commonly heavy mortality (estimated to be 70%) due to high predation by carnivorous fishes previously present in ponds (Aquatic Farms, 1986).

Before 1980 fingerling availability was the major constraint to fish culture in Bangladesh (Aquatic Farms, 1986) and those ponds which were stocked obtained their fry or fingerlings only from natural sources. Fry are available in very large numbers in certain well known areas in the major rivers. This source of stock has recently been supplemented from government and, increasingly, private nurseries and hatcheries. The commonly farmed species, catla (Catla catla), and rui (Labeo rohita), mrigal (Cirrhina mrigala) and kalbaus (Labeo calbasu) are now being supplemented with "exotic" carp species which, in a polyculture system, increases the efficiency of utilization of the available feed in the pond (FAO/UNDP, 1985). Along with major and Chinese carps in composite culture, common carp grow well with the advantage that each species utilizes a different ecological niche in the pond (Chaudhuri et al., 1975; Jhingran, 1977).

The "exotic" carps so far introduced comprise silver carp (Hypophthalmicthys molitrix), common or mirror carp (Cyrpinus carpio), grass carp (Ctenopharyngodon idella) and to a lesser extent, bighead carp (Aristichthys nobilis) and the black carp (Mylopharyngodon piceus) (FAO/UNDP, 1985). Both scaled and mirror varieties of common carp were

introduced to Bangladesh during the 1960s and early 1970s. Since their introduction common carp have gained wide acceptance throughout the country because of the ease of spawning in ponds and because it can tolerate a wide range of environmental conditions.

Stocking ponds with hatchery reared fry and fingerlings is preferable because it enables the farmers to select the species and the stocking ratios in which they are presented. Wild stock is less suitable because it is difficult to distinguish the various species of carp fry from one another or from other less desirable or predatory species.

Bangladesh currently has approximately 1.3 million ponds which comprise a total area of about 151,000 hectares (Aquatic Farms, 1986). Most of the ponds were not constructed specially for fish culture, but rather are borrow pits from which landfill was taken to elevate residential areas above the flood level or were built as water storage ponds for domestic and agricultural uses. Ponds are divided into "Cultured" -those which are artificially stocked and do not flood, "Cultivable" -those which are in good repair but which have ownership problems and are 'wild' stocked, and "Derelict" - ponds which dry up or are otherwise overgrown and neglected or need construction of embankments to prevent flooding (FAO/UNDP, 1985). Of the existing ponds only 45.7% are cultured, 30.1% are cultivable and the remainder are derelict. Ponds range from 0.2 hectare to more than 20 hectares in area. The average annual productivity of cultured ponds is about 1160 kg/ha.

Carps are also stocked into larger water bodies such as some beels, haors and baors, together with Kaptai Lake. The most successful attempt

to increase the productivity of larger water bodies in Bangladesh has been the World Bank Oxbow Lake Fishery Project in Jessore. The productivity of one of the six selected baors (Baluhar) has now increased from 450 to 900 kg/hectare in 1984/85 by stocking 3 inch fingerlings (FAO/UNDP, 1985).

Recent rapid expansion of the private sector carp spawn and fingerling production in Bangladesh has effectively removed fingerling availability as a constraint for most farmers. There are two types of fingerling production: the multi-cropped intensive culture and the single crop extensive method. Most farmers practice multi-crop intensive culture to rear fry to fingerling stage (Aquatic Farms, 1986). Farmers use a two stage growing system, first stocking their spawn in small nursery ponds (about 1000m^2 or less) and transferring them, after about two weeks, to larger rearing ponds. To eliminate predacious insects before stocking spawn, farmers use multiple netting with very small mesh nets. Farmers can grow as many as four crops from a single rearing pond in areas where hatchery spawn is available (Aquatic Farms, 1986). Farmers using the more extensive single stage rearing are not full-time fish farmers and stock their ponds just once in a season. They begin to harvest as the fingerlings reach 5-7 cm, and continue until most of the fish are sold and use their pond for food fish production until next season using whatever fish remain.

There is no real consensus on the rates or proper methods of feeding and fertilization. Mustard oilcake and cow manure are probably the most widely used inputs, but application rates are highly variable. Most

farmers apply a single dose of manure of 2000-4000 kg/ha, usually from their own livestock, and apply the mustard oilcake in one or two applications per week at 20-25 kg/ha per application (Aquatic Farms, 1986). Triple super phosphate (TSP), urea and potash are used but are not widely or consistently available. Some farmers use wheat or rice bran with mustard oilcake.

The main constraint to future development of fish culture in Bangladesh appears to be lack of an adequate fisheries extension programme. This is required to disseminate improved aquaculture technology to village level pond farmers on subjects including proper stocking rates, feed and fertilization techniques. Although the average annual productivity of cultured ponds is about 1160 kg/hectare, levels of 3000-4000 kg/hectare have been achieved through the adoption of improved pond culture practices at Raipur hatchery (FAO/UNDP, 1985).

1.3.1 Requirement for feeds and feedstuffs for aquaculture in Bangladesh

As aquaculture practices become more intensive in order to increase production, the needs for fertilizer and feed increase, whether the animal being cultured is carp, shrimp or fresh water prawn. FAO/UNDP (1985) in its 20 year Fishery Development Plan for Bangladesh has set fish production targets of 1.12 million tonnes by the year 2005 from the existing 0.77 million tonnes. To meet the target, techniques for intensification of aquaculture including improvements in stocking, feeding, fertilization, water management, cage culture, feed formulation and

TABLE 1.5

Summary of feed, fertilizer and chemical needs for 2005

TYPE	MT/Year
Rotenone	9,200
TSP (Triple Super phosphate)	25,200
Urea	24,300
Lime	117,875
Cow dung	668,500
Rice bran	78,300
Mustard oilcake	80,400
Compound shrimp feed	32,400
Compound prawn feed	12,500

Adapted from FAO/UNDP, 1985

production, aeration and pumping, pond and other enclosure construction, engineering, nutrition, and pathology are essential.

The expansion of aquaculture proposed for Bangladesh over the next 20 years (FAO/UNDP, 1985) will create a significant demand for feeds, fertilizers and chemicals. There is already a shortage of livestock feed in Bangladesh and the amounts available for shrimp and freshwater prawn in particular will be inadequate well before the year 2005. It will eventually be necessary to import some of the high protein components, but meantime efforts should be made to develop local sources of feed. For this purpose, there are many alternative materials which might be utilized if available. It is therefore, impossible to be exact about the requirement for each commodity. However, FAO/UNDP (1985) has made an attempt to estimate the requirements by making some assumptions about the types and quantity of feed, fertilizers and chemicals needed for targeted aquaculture production in the year 2005 as is shown in Table 1.5.

1.3.2. Availability of feeds and feedstuffs in Bangladesh

Presently in Bangladesh limited quantities of fish meal are available. However, it has been estimated that 25,000 tonnes of trash fish per year are currently discarded at sea. If brought ashore, this could be used for fish meal production or, if used while fresh, made into moist feeds. Twenty five thousand tonnes of trash fish could yield up to 5000 tonnes of fish meal, together with other products (FAO/UNDP, 1985). Additionally, the waste heads and shells from shrimp processing units

could be used in moist feeds or dried to form shrimp meal. Shrimp waste is a valuable ingredient for shrimp diets (New and Singholka, 1982). The quantity of shrimp waste available will rise proportionately with the growth of shrimp aquaculture.

Currently, there are only two medium size feed mills in Bangladesh (Monno Grimixpel Ltd and Bangladesh Solvent Oil Ltd). Both have pelleting equipment and their combined capacity is estimated to be 8000 tonnes/year. Presently, the operation is not at full capacity because of lower demand. Shrimp and prawn feed requirements for 2005 will be about six times the estimated capacity in 1985 (FAO/UNDP, 1985). In view of shortages of fish meal in Bangladesh, it will be necessary to import either compounded shrimp and prawn feed or raw materials with which to produce them.

Presently, Bangladesh is producing sufficient rice bran to meet the demand for livestock and aquaculture. In 1982/83 nearly 960,000 tonnes of rice bran was produced locally (BBS, 1984). A large quantity of oil crops are produced in Bangladesh. The oils are mainly used for cooking and industrial purposes. After extraction of oil, the seed cakes are used principally for livestock feeds and some are exported. The total production of oilseed crops in Bangladesh in the year 1984-85 is shown in Table 1.6. The estimated quantity of all types of oilseed cakes produced was 150,000 tonnes. The projected production of oilcakes in the year 2005 would be much higher. If the government imposes a ban on the export of oilcakes and the demand for the livestock remains the same, the local supply of oilcakes could meet the demands of aquaculture.

TABLE 1.6

Oil crops in Bangladesh

CROPS	USE	ACREAGE (000 acres)	PRODUCTION (000 tonnes)
1. Rapeseed (*Brassica campestris*)	Edible purpose (cooking)	464	129
2. Mustard (*B. juncea*)	Edible purpose (cooking)		
3. Sesame (*Sesamum indicum*)	Edible (cooking), hair oil, seeds are directly used as an ingredient of confectionary food items	98	22
4. Groundnut (*Arachis hypogaea*)	Roasted nuts, confectionary purpose	50	21
5. Linseed (*Linum usitatissimum*)	Industrial purpose (for making paints, varnishes)	36	8
6. Coconut (*Cocos nucifera*)	Hair oil, cooking, directly as ingredients of food	76	82
7. Castor (*Ricinus communis*)	Industrial (varnish, soap making, lubricants etc) and medicinal purpose	1	0.3
8. Niger (*Guizotia abyssinica*)	Cooking, soap making and lubricants	2	0.3
9. Safflower (*Carthamus tinctorius*)	Edible (cooking)		
10. Sunflower (*Hellanthus annus*)	Edible (cooking)		
11. Soybean (*Glycine max*)	Seeds are used for making confectionary items		

Source of statistical data: Statistical Year Book of Bangladesh 1984-85, Bangladesh Bureau of Statistics

Alternative feeds should also be identified and brought into production to achieve the targets set for aquaculture production. Substantial quantities of potentially useful ingredients such as meat wastes, blood, frog-wastes and cotton seed are currently discarded because of lack of demand or processing ability (FAO/UNDP, 1985). The importation of high protein feed ingredients for aquaculture to produce fish for local consumption would be too expensive and should be avoided.

1.3.3. Selection of various oilseed cakes as alternative protein sources to fish meal

In recent years the intensification of carp fry production in Bangladesh has made it essential to develop suitable complete and supplemental diets for use in hatcheries and nurseries. At present fish meal supplies a major proportion of dietary protein in commercial fish feeds (Tacon and Jackson, 1985). Moreover, the starter/fingerling ration of many fish species often contains even higher dietary inclusion levels of fish meal (up to 70% by weight, Tacon, 1981). However, the use of fish meal in diets for fry and fingerlings in Bangladesh is not feasible because it is not widely available and too expensive. Therefore, it is necessary to find alternative protein sources to develop suitable diets for carp fry and fingerlings to increase aquaculture production.

Of the feed ingredients available in Bangladesh, the most promising alternatives to fish meal in carp fry diets are the oilseed cakes or meals namely mustard oilcake, (Brassica juncea), linseed meal (Linum usitatissimum) and sesame meal (Sesamum indicum). The average protein content of various oilseed meals is generally high. The protein content

in mustard oilcake and linseed meal varies between 30-40% and in sesame meal 35-45% on a dry basis. Both linseed and sesame meal are high in the essential amino acid methionine (ADCP, 1983).

The efficacy of various alternative protein sources as partial or complete replacement for fish meal in carp has, to some extent, been evaluated (Viola et al., 1982; Atack et al., 1979; Abel et al., 1984; Dabrowski et al., 1982). However, information on the use of mustard, linseed or sesame meal as alternative protein sources in carp diets is scanty (Hasan, 1986). Mustard oilcake is presently used as a supplemental feed in carp hatcheries and nurseries. It is therefore necessary to evaluate the quality and suitability of these oilcakes as ingredients of carp diets for Bangladesh. These three have therefore been selected for further consideration.

1.4 IMPORTANT ANTI-NUTRITIONAL FACTORS IN THE SELECTED FEED INGREDIENTS

1.4.1 Glucosinolates

Many cruciferous plants, such as Rapeseed (Brassica napus) and Mustard seed (B. juncea), contain thioglucosides (also referred to as glucosinolates) which are goitrogenic and growth depressant (Liener, 1977). Goitrogens are natural products which cause hypothyroidism with an enlargement of thyroid when consumed by man and animals (Salunkhe and Wu, 1977). In 1980, Bussy established the relationship between the

mustard oil allyl isothiocyanate and its precursor "sinigrin" when he isolated the latter by aqueous extraction of preheated black mustard seeds (B. nigra) (Bell and Belzile, 1965). This clearly established the simulatenous presence of a thioglucoside and a thioglucosidase (enzyme) in the same seed.

Brassica species are not identical with respect to their glucosinolate contents. Brassica juncea (brown and oriental mustard) just like other mustard seeds (for example, B. hitra or white/yellow mustard and B. nigra or black mustard) is characterized by the presence of only one glucosinolate, (sinigrin) in contrast to the complex situation in B. camprestris and B. napus which contain several glucosinolates (Maheshwari et al., 1981 and Shah et al., 1977). The major glucosinolates in the latter species are gluconapin and progoitrin and to some extent glucobrassicanapin. The allyl isothiocynate from sinigrin is easily recognised because of pungency, a characteristic of mustard oils which has long been recognised.

The intact glucosinolates are apparently free of toxicity but on hydrolysis by an endogenous enzyme (myrosinase) present in seed and unheated meal, these compounds yield undesirable and potentially toxic products such as isothiocyanates and thiocyanates, nitriles and oxozalidinethiones (Bell and Belzile, 1965; Slinger, 1977; Van Etten, 1969; Liener, 1975). The glucosinolates of Brassica sp. and other crucifers are associated with the enzyme myrosinase which hydrolyses them to glucose, bisulfate and an unstable organic aglucone when wet unheated material is crushed. The organic aglucone portion of the molecule is

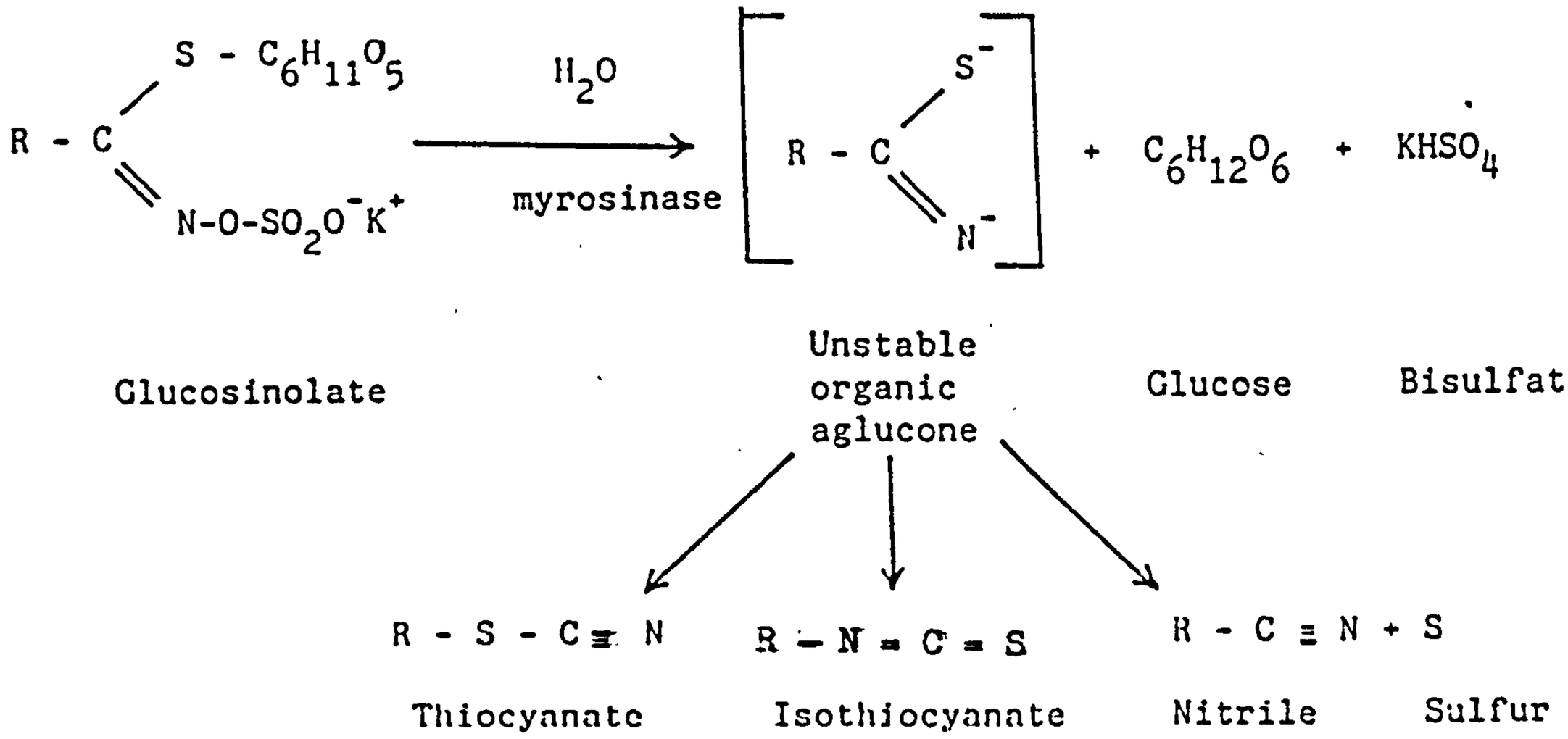


FIGURE 1.1 Enzymatic hydrolysis of glucosinolate

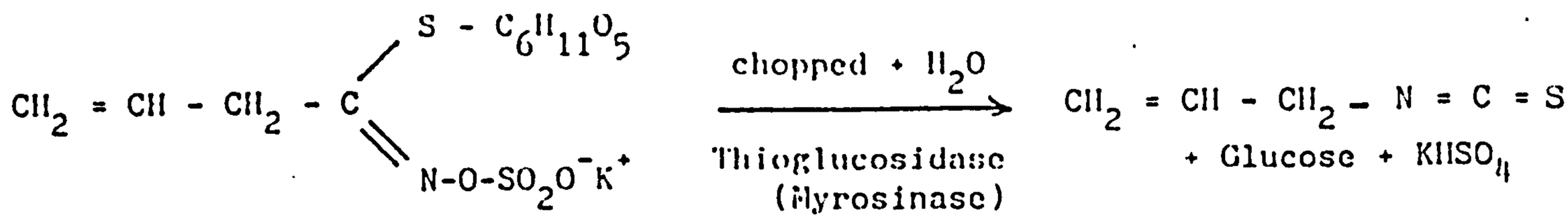


FIGURE 1.2 The enzymatic hydrolysis of allyl thioglucoside (sinigrin) to allyl isothiocyanate

TABLE 1.7

Different types and levels of glucosinolates in mustard seed

Types of Mustard Seed	Types of glucosinolates		Authors
	Sinigrin (Allyl isothiocyanate)	Gluconapin (Butenyl isothiocyanate)	
1. Mustard seed meal (*Brassica juncea*)	6.2 mg/g	-	Marangos & Hill (1976)
2. Mustard seed (whole)	9.16 "	-	Mustakas *et al.* (1963)
(Steam meal)	0.04 "		
3. Mustard oilcake		-	Shah *et al.* (1977)
B. juncea (Purabi Raya)	9.40 "	-	
B. juncea (S-9)	6.00 "		
4. Oriental mustard *B. juncea*	4.00 "	-	Mustakas *et al.* (1962)
5. Mustard seed *B. juncea*	Principal	Minor	Liener (1977)
6. Mustard meal *B. juncea*	Major	Minor	Bell *et al.* (1984)
7. Mustard seed meal	Major	-	Maheswari *et al.* (1981)

usually converted to thiocyanate, to nitriles and sulfur or through a "lossen" rearrangement to isothicyanate (Van Etten and Wolff, 1973). The basic structure of glucosinolate which was revised in 1965 and firmly established by Ettlinger and Lundeen (1957) is shown in Figure 1.1. The enzymatic hydrolysis of allyl thioglucoside (sinigrin) to allyl isothiocyanate is shown in Figure 1.2.

These hydrolytic products of glucosinolates when ingested by rats or other experimental animals produce various adverse affects ranging from depressed growth, loss in weight, enlarged thyroid and kidney to death in rats (Srivastava et al., 1975; Nishie and Daxenbichler, 1980; Marangos and Hill, 1976). Similar growth depression and thyroid enlargements are also reported in fish fed with rapeseed and mustard oilcakes (Dabrowski and Kozlowska, 1981; Dabrowski et al., 1982; Higgs et al., 1982; Yurkowski et al., 1978; Hardy and Sullivan, 1983; and Capper et al., 1982). The thiocyanate, isothiocyanates and nitriles prevent the thyroid from accumulating iodine. The different types of levels of glucosinolates present in mustard seed/meals are shown in Table 1.7.

1.4.2 Phytic acid

Phytic acid is a major component of all plant seed constituting 1-3% by weight of many cereals and oilseeds although as high as 3-6% has been reported for particular varieties (Graf, 1983 and Cheryan, 1980). Typical phytic acid contents of some cereals and oilseeds are presented in Table 1.8. Phytic acid forms strong complexes with protein,

TABLE 1.8

Typical phytic acid contents of some cereals and oilseeds

SAMPLE	PHYTIC ACID (%) (Dry basis)	REFERENCE
Peanut meal (defatted)	1.7	
Rapeseed meal (defatted)	3.69	Cheryan (1980)
Sesame meal (defatted)	5.18	
Sunflower seeds	1.9	
Lima beans	2.5	Graf (1983)
Dehulled sesame seed	5.3	
Soybean	1.4	
Sesame meal dehulled	3.6	Erdman (1979)
Cotton seed flour glanded	2.9	
Linseed	4.2	Madhusudhan & Singh (1983)
Soybean (mature)	1.78	Welch & House (1982)

phosphorous, zinc, calcium and magnesium and other polyvalent minerals at intestinal levels of pH found in fishes and other monograstrics that have little phytase (Erdman, 1979). Phytic acid in most cereals (for example corn, wheat and rice) is associated with specific parts such as the endosperm, germ and hull. In contrast phytates are found throughout the kernel of oilseeds.

The interaction between phytic acid, mineral and/or protein appears to be the primary factor responsible for its adverse nutritional effects in high phytate diets. The mechanism of this interaction can be explained by the structure of phytic acid. The chemical structure of phytic acid, the hexaphosphate of myoinositol, has been proposed by Anderson (1914) and as shown in Figure 1.3 is probably the correct one. Neuberg (1908) had earlier proposed a slightly different structure given by the formula $C_6H_{24}O_{27}P_6$ distinguished mainly by having three P - O - P linkages between pairs of adjacent phosphates (shown in Figure 1.4).

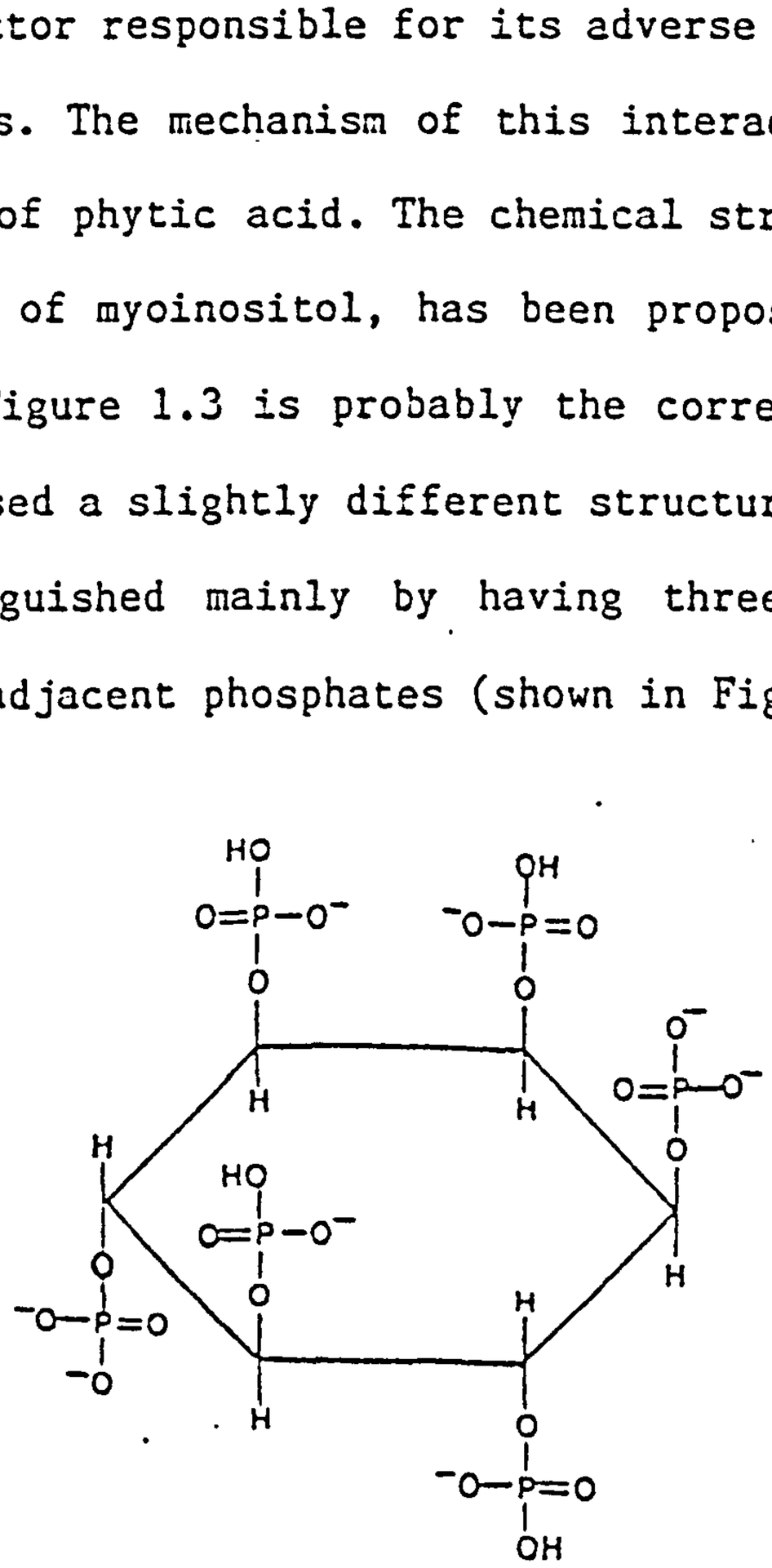


FIGURE 1.3: Structure of phytic acid

(proposed by Anderson, 1914)

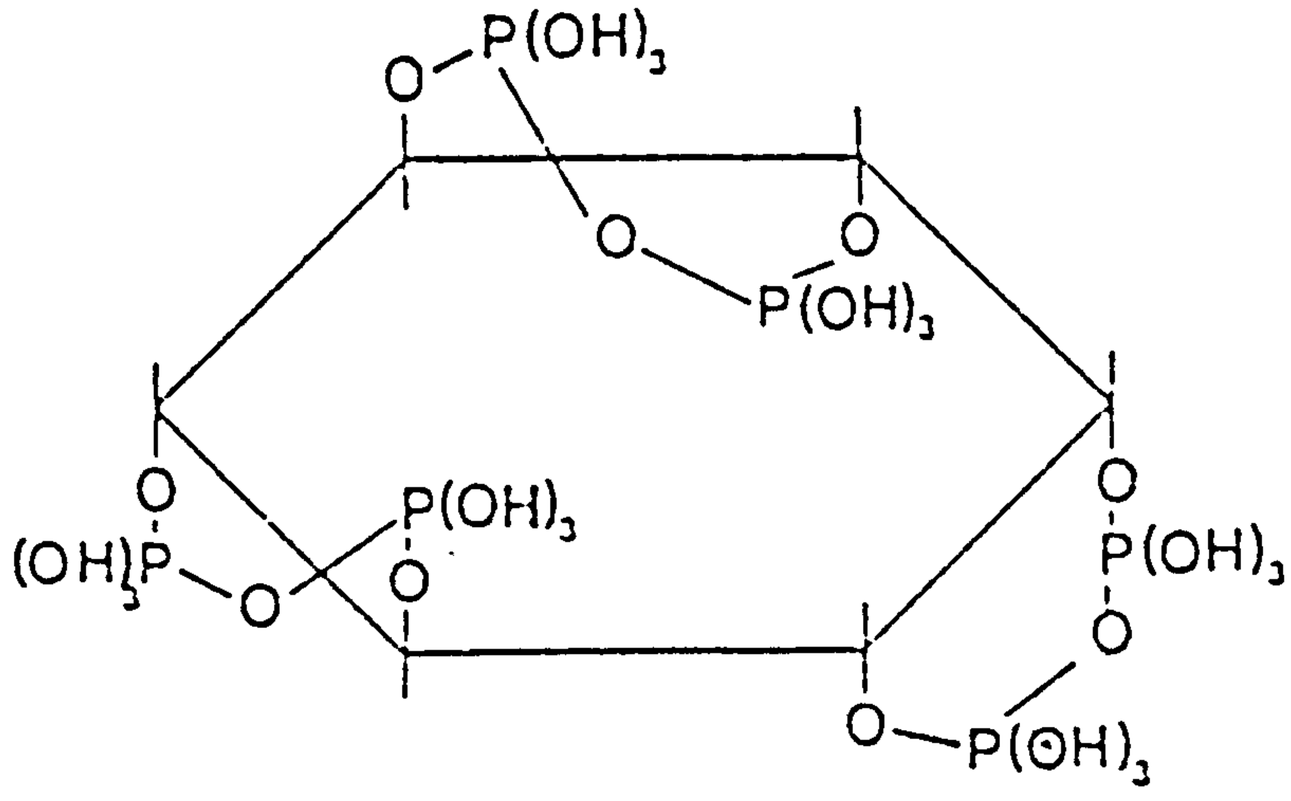


FIGURE 1.4: Structure of phytic acid
(Suggested by Neuberg, 1908)

A number of conflicting conclusions about the precise structure of phytic acid might be due to uncertainty of assay procedures, source of raw materials and methods of purification. Phytic acid itself is thought to be unstable and is most frequently experimentally used in the form of the sodium salt. Nevertheless, current opinion in the literature appears to favour Anderson's structure since many of the physiochemical properties, interactions and nutritional effects can be better explained with this structure. Thus, on the basis of the Anderson structure, the proper name for phytic acid is myoinositol - 1,2,3,4,5,6 - hexakis dihydrogen phosphate (IUPAC-IUB, 1968).

Due to its multiplicity of reactive phosphate groups, phytic acid can complex a cation with a phosphate group itself, between two phosphate groups of a molecule or between phosphate groups of different phytic acid molecules (Gosselin and Coghlan, 1953). Thus, phytic acid probably fits the classic definition of a chelating agent compound.

1.4.3. Cyanogenetic glucosides

The cyanogenetic glucosides are compounds that yield hydrogen cyanide upon treatment with acid or an appropriate hydrolytic enzyme (Salunkhe and Wu, 1977). The glucoside itself is not toxic but the hydrocyanic acid (HNC) which is released upon hydrolysis by an endogenous enzyme may be toxic. Plants which contain high levels of such cyanogenetic glucosides, and are commonly eaten by man or domestic animals, include cassava, lima beans, sorghum and linseed. Linseed meal has been reported to contain as high as 53 mg/100g of HCN (Madhusudhan and Singh, 1983; Montgomery, 1964).

The toxic principle in lima bean, cassava and linseed is an identical CN - containing glucoside (phaseolunatin or linamarin) from which acetone and HCN are released by heat labile autoenzymes (Figure 1.5) provided that the cellular structure is disrupted by bruising or crushing (Montgomery, 1964). Linamarin, a cyanogenetic glucoside in linseed, is toxic to fish (Tunison and McCay, 1938).

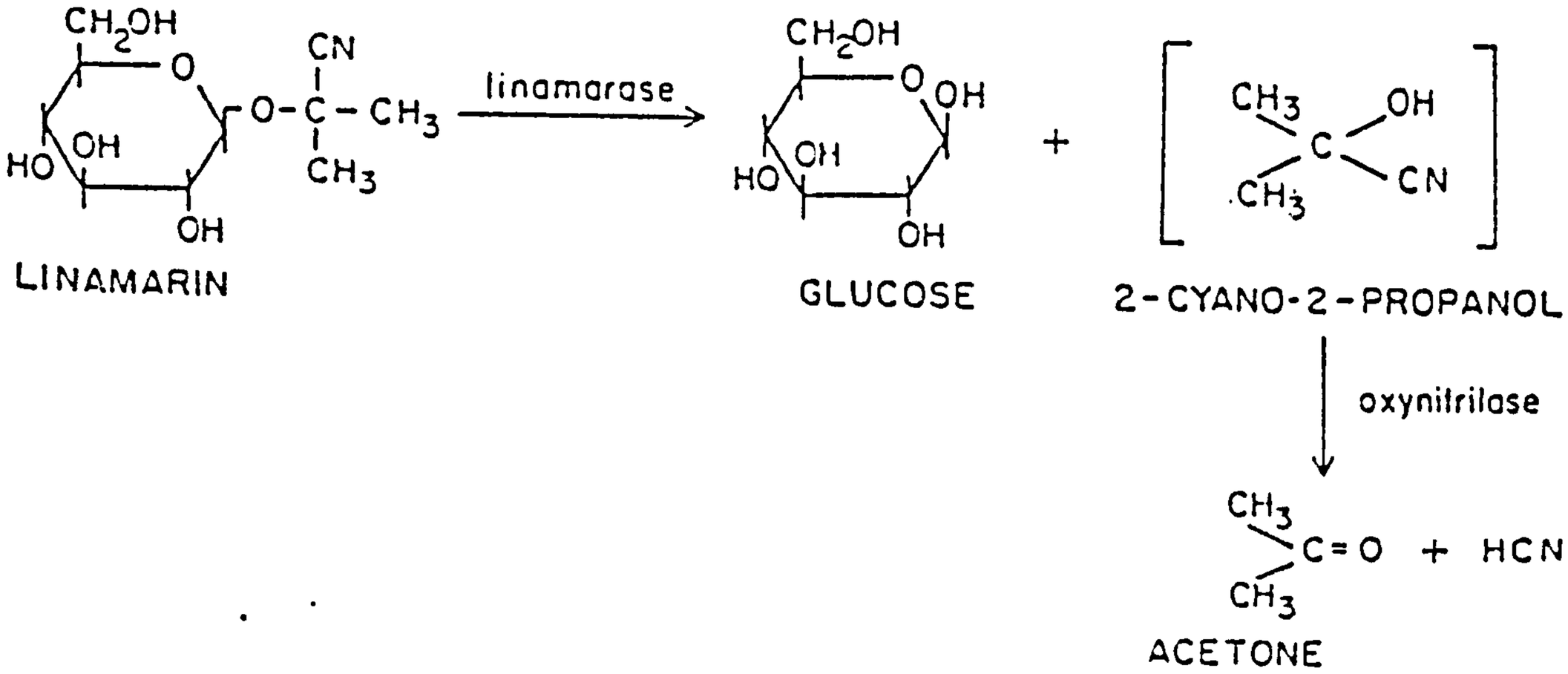


FIGURE 1.5 The enzymatic hydrolysis of linamarin, the cyanogenetic glucoside of linseed

A reduction in toxicity can be accomplished by different processing methods - (soaking, boiling, roasting, sundrying) which serve to inactivate the enzyme and volatilize any HCN that may have been released.

1.4.4. Tannin

As defined by Singleton and Kratzer (1973), a tannin is any polyphenolic substance that has a molecular weight greater than 500. Two distinctive groups are the hydrolysable and the condensed tannins. In earlier literature there was considerable confusion as to the nature of substances designated as "tannin". Thus, Valaer (1941) in referring to tannic acid, stated that "tannic acid, gallotannic acid, tannin and

digallic acid are terms used interchangeably for the same substance". It has been reported that rapeseed meal contains up to 3.7% of tannins (Clandinin and Heard, 1968; Yapar and Clandinin, 1972). Capper et al. (1982) found 1.44% and 1.49% of tannic acid in unroasted and roasted mustard oilcake respectively.

Tannins have been extensively investigated because of their adverse effects on growth of animals (Glick and Joslyn, 1970) and poultry (Chang and Fuller, 1964; Vohra et al., 1966; Marquardt et al., 1977); protein utilization (Vohra et al., 1966) and metabolizable energy value of feed (Yapar and Clandinin, 1972).

1.5 THE AIMS OF THE RESEARCH

From the foregoing discussion it is clear that one of the major problems faced by rapidly growing aquaculture in many developing countries, including Bangladesh, is the availability of feeds, especially for fry and fingerlings. Thus, the aims of the present research were to study:

(i) The nutritive value of mustard oilcake, linseed and sesame meal as alternative protein sources in the diet of common carp;

(ii) The effect of dietary amino acid supplementation of diets containing these oilcakes/meals on the growth performance and food utilization of common carp;

(iii) The digestibility of protein and amino acids of these protein sources in the diet of common carp;

(iv) The effect of different processing methods on the nutritive values and possible destruction of anti-nutritional factors in the feed ingredients;

(v) The use of anti-nutrients in purified diets to determine their effects on growth performance of common carp as compared with diets containing the same quantity of anti-nutrients contributed by the plant protein sources under test;

(vi) The histopathological changes in fish tissues resulting from ingestion of the various anti-nutrients under study.

CHAPTER 2 : GENERAL MATERIALS AND METHODS

2.1 EXPERIMENTAL FACILITIES

All experiments were conducted in one of two different types of recirculated water system.

2.1.1. System for Growth Trial

This system consisted of two identical recirculating units. Each unit consisted of thirty two 10 litre polypropylene experimental tanks (Figure 2.1 and 2.1a). Water was supplied to these tanks (1 l/min/tank) from a 125 l header tank through a common inflow pipe. Water in the header tank was aerated (using airstones) and heated (using 240 volt, 200 watt aquarium heaters) to maintain a temperature of 27° ±1°C throughout the experimental period. Water was tangentially jetted into the experimental tanks both to increase aeration and to induce a circular flow. Because of this circular flow, the tanks were self cleaning. The central stand pipe drainage system of the experimental tank is shown in Figure 2.1b. Over each stand pipe a sleeve was fitted with a number of holes at the bottom, so that faeces and uneaten food were sucked from the tank bottom through the holes and into the outflow pipe. The outflow water from the experimental tanks drained through the stand pipe and collected in 3cm internal diameter drain pipes and was carried, by gravity, to a gravel filter tray, placed over first of the three settling/biological filter tanks (125 l).

These biological filter tanks contained a plastic ring medium (Mass Transfer Ltd, Hobsons Lane, Cumbria) which promoted settling by

FIGURE 2.1a Diagrammatic representation of the recirculated water system used for the growth trial in all the experiments

a. Inflow pipe into the experimental tanks

b. Experimental tanks

c. A circular tank containing filter wool, gravel and oyster shells

d. Outflow drain pipe

e. Biological filter tank

f. Sump tank

g. Pump

h. Header tank

i. Pipe connecting pump and header tank

j. Overflow pipe

⟶ Direction of water flow

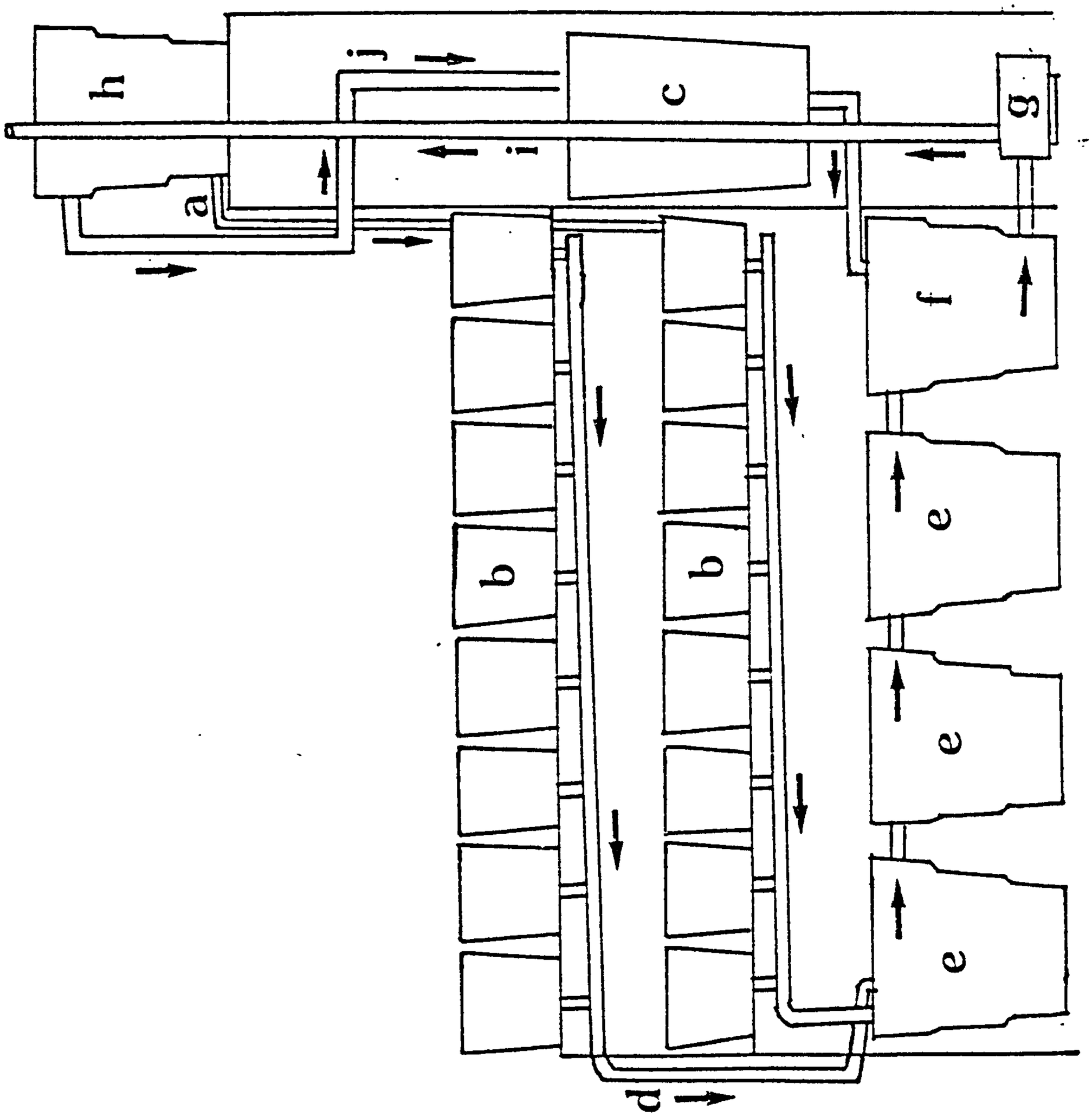


FIGURE 2.1.a

FIGURE 2.1b Diagrammatic representation of one of the experimental tanks in recirculated water system used in growth trial

a. Tank lid
b. Inflow pipe
c. Fine mesh covering
d. Outer jacket of stand pipe
e. Stand pipe
f. Water outflow to drain

⟶ Direction of water flow

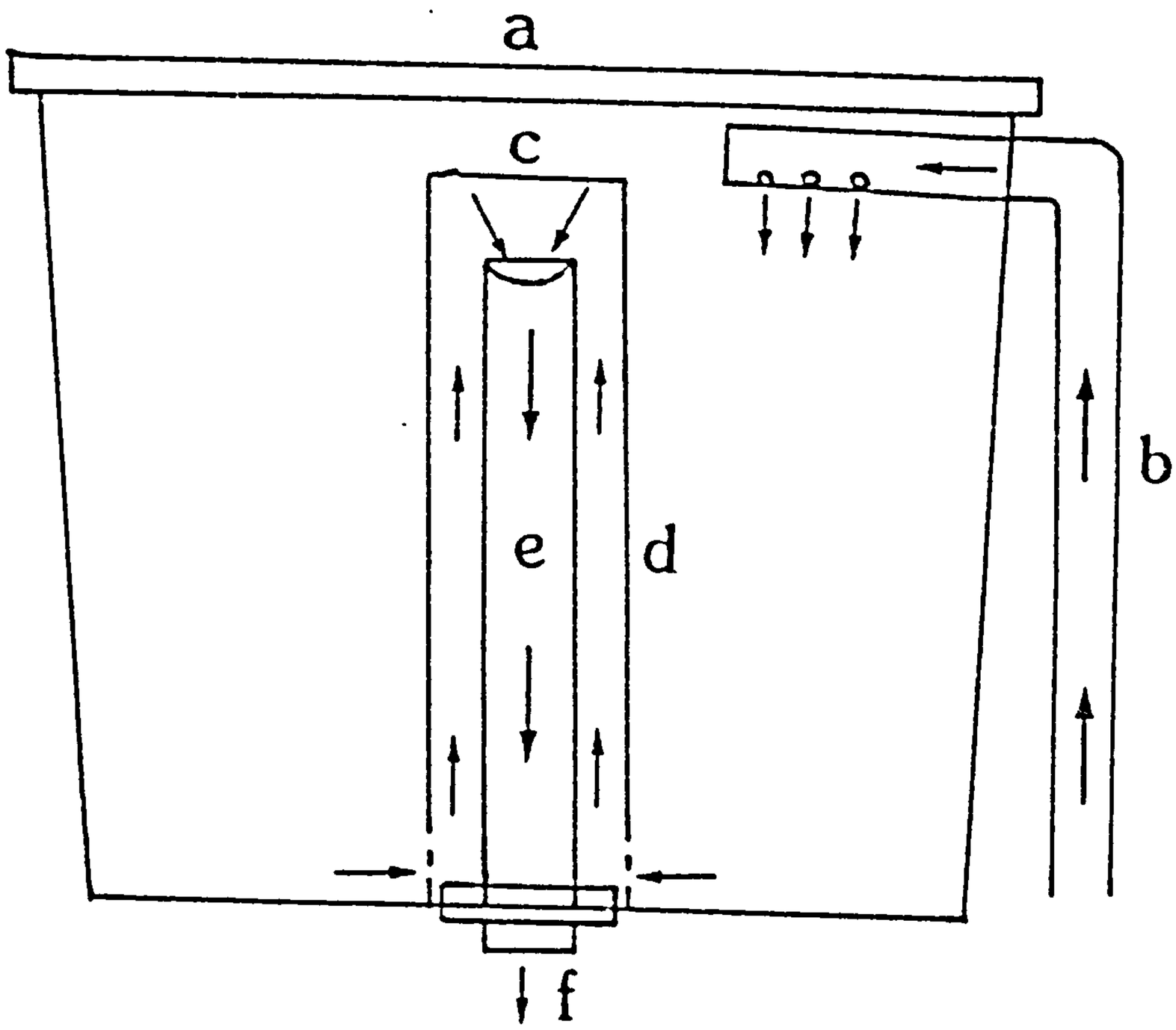


FIGURE 2.1.b

increasing retention time and provided a substrate for attachment of nitrifying bacteria. Water flowed through the three settling tanks under gravity before overflowing into a 125 l sump tank. Water was pumped from here (using a Beresford Centrifugal pump) to the header tank for distribution into the experimental tanks. Excess water from the header tank overflowed to the sump tank through glass wool filters, fine gravel and Oyster shell filters designed to trap suspended solid wastes (Figure 2.1a) and increase the hardness to buffer pH. A high degree of recirculation was maintained with minimal replacement of evaporation and splashing losses by fresh water make-up. A constant photoperiod of 12 hours light and 12 hours dark was maintained. All experimental tanks and header tanks were supported on framing of aluminium alloy 'handy angle'. The experimental system was constructed in the Tropical Aquarium Building of the Institute of Aquaculture in which the air is maintained at an ambient temperature of 25°-28°C.

2.1.2. Faecal Collection System

This system consisted of one recirculating unit having sixteen 10 l circular polypropylene experimental tanks. This system differed from that used for growth trials by having had the tank bottoms removed and large polypropylene funnels of appropriate diameter attached by silicone rubber adhesive (Fig. 2.2 and 2.2a). To the bottom end of this funnel was attached a clamped rubber drain tube. Periodic release of this clip allowed collection of deposited faeces. The mouth of the conical tank bottom was screened with 10mm square plastic mesh to exclude fish from the faecal collection zone, but allow faeces to pass through and settle

at the bottom.

During feeding a disc of perspex of appropriate diameter was placed over the netlon to allow fish to feed (by preventing the pellets from passing into the faecal collection zone). About 15 minutes after feeding this disc was removed and about 1 litre of water was flushed away from each tank so that any uneaten food or faeces was removed. Water was supplied to the tanks (1 l/min/tank) from the header tank in which water was aerated (using airstones) and heated (using an Armitage Nimrod heater of 240 volts, 200 watts) to maintain a temperature of 27° ±1°C (as in the growth trial system). Outflow from the tanks was via outlet pipes 3cm below the tank surface into a collecting duct which led to the first of three 125l settling/biological filtration tanks (details shown in Fig. 2.2, 2.2a and 2.2b). Water then passed into a sump tank from which it was pumped to the header tank. Excess water from the header tank returned to the sump through the overflow pipe. Adequate oxygen supply was ensured by airstones in each of the experimental tanks.

The water quality in both the system during the experimental periods was monitored weekly and ranges of results are shown below:

Temperature	26°-28°C
Dissolved oxygen	6-8 mgl^{-1}
Total ammonia	0.1-0.54 mgl^{-1}
pH	6.6-7.5

FIGURE 2.2a Diagrammatic representation of faecal collection system

a. Inflow pipe

b. Experimental tank

c. A circular tank containing filter wool, gravel and oyster shells

d. Outflow drain pipe

e. Biological filter tank

f. Sump tank

g. Pump

h. Header tank

i. Pipe connecting pump and header tank

j. Overflow pipe

⟶ Direction of water flow

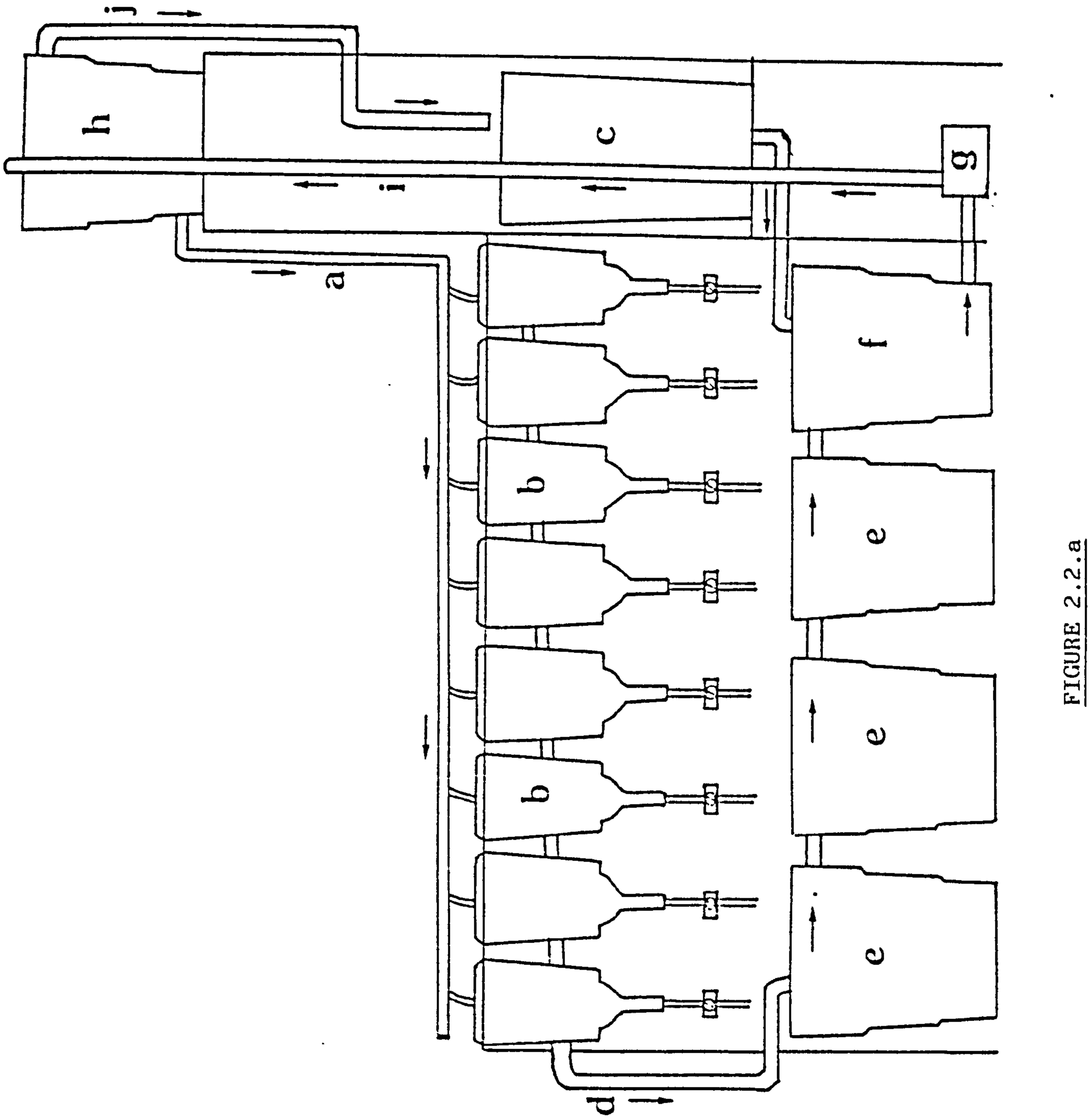


FIGURE 2.2.a

FIGURE 2.2b Diagrammatic representation of one of the tanks used in faecal collection system

a. Inflow pipe
b. Water flow control valve
c. Medium mesh size netlon net
d. Outflow pipe
e. Rubber tubing
f. Stop cock arrangement for faeces collection

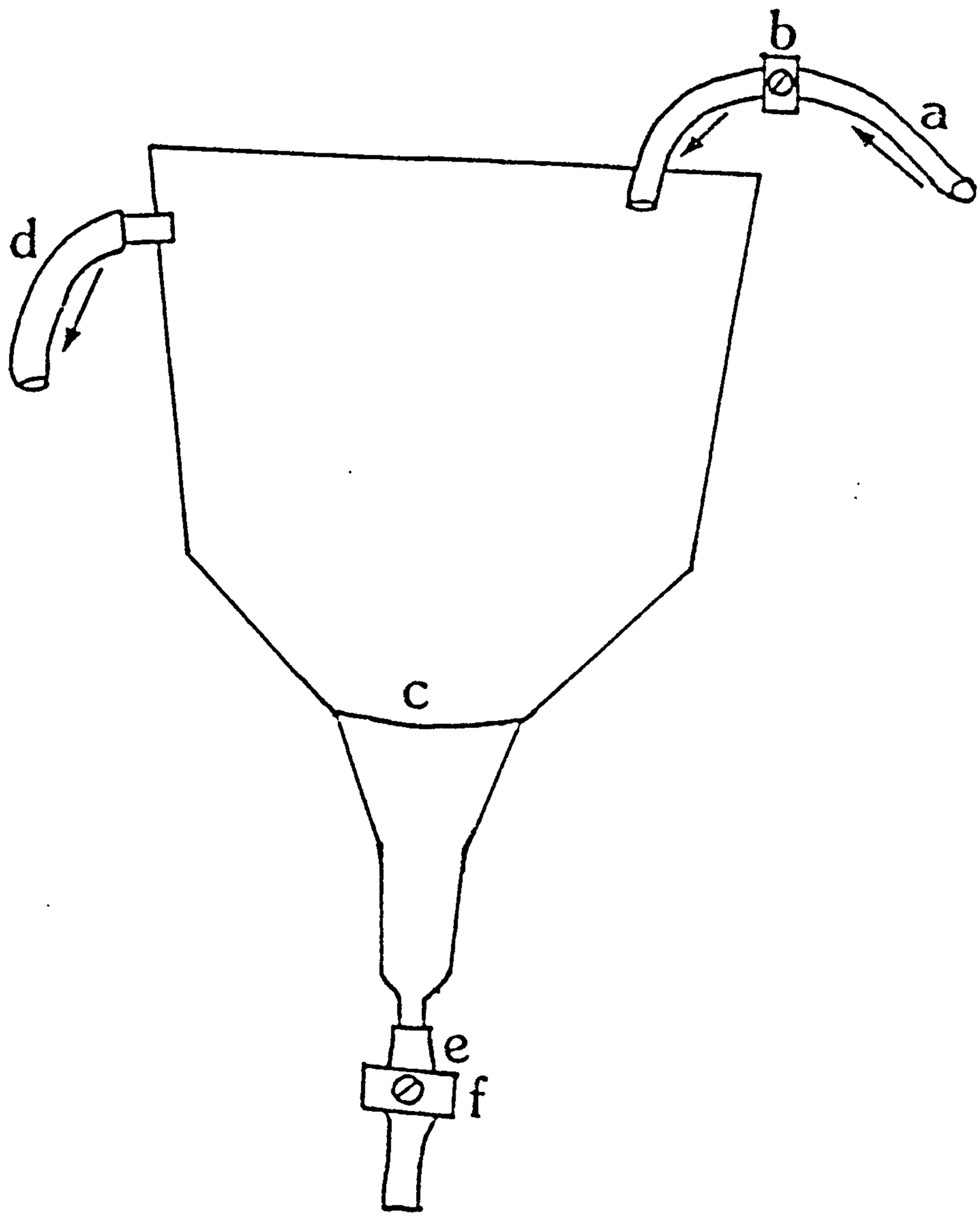


FIGURE 2.2.b

2.2.1. Experimental Animals

Common carp fry of mirror variety from various sources were obtained for the different experiments. All the fry obtained were induced bred, in warm water (20°-24°C) recirculatory systems. The fry supplied were derived from various fish farms in England (Table 2.1). They were brought to Stirling, by rail, packed in sealed polythene bags containing water and oxygen. There was no mortality during transport.

2.2.2. Quarantine Procedure

Upon arrival each batch of fish fry was kept in an isolated constant temperature room in the restricted quarantine area of the Institute of Aquaculture. Fish were kept in quarantine for 2-3 weeks and examined for the presence of parasites, bacteria and viruses. During quarantine they were held in 80 l glass aquaria, serviced by an "Eheim" combined water pump and filter through which water was continuously circulated. The water temperature was 15°C. Fish fry were fed with commercial trout pellet (Ewos-Baker's Omega No.3; protein 49%).

The fish obtained from Trent Eel Plc (used in Experiment 6) required treatment for "Ich" (Icthyopthirius multifilis) infection. A combined bath treatment of malachite green (0.5ppm) and formalin (50ppm) was given for 30 minutes for 3 alternate days. At the time of treatment, all the tanks including "Eheim" pumps were cleaned and washed. After the quarantine period had elapsed and two days before transferring fish to Tropical Aquarium Building, the temperature of the tanks was increased

TABLE 2.1

Various sources of fry supply used in different experiments

Name and Address of fish farms	Size of fish obtained (g)	No. of fish obtained	Fish used in experiments
New Hay Fisheries Ltd Clifle Selby Yorkshire, England	0.5 - 1.0	1500	1, 2, 3
Humberside Fisheries Cleaves Farm Skerene Driffield, England	1.0 - 1.5	1000	4.1, 4.2
Calverton Fish Farm Severn Trent Water Authority Nottingham, England	1.0 - 2.0	1000	5.1, 5.2
Trent Eel Plc Ratcliffe-on-Soar Power Station Nottingham, England	1.5 - 2.5	500	6

(using aquarium heaters 240 volts, 200 watts) to about 27°C. Fish were then either transferred to the experimental systems or were maintained in 200 l stocking tanks in a recirculating water system (28°C) in the Tropical Aquarium Building.

2.2.3. Acclimation and Weighing Procedures

One week before the start of each experiment fish were transferred to the experimental tanks from stocking tanks for acclimation. Fish were fed with trout pellet (Ewos Baker's Omega No.3) during this period. For initial and final samples, all fish were individually weighed under anaesthesia with Benzocaine (50 mg/l) solution (Ross and Geddes, 1979). Fish were netted, drained of water and gently blotted on a soft paper towel before individual weighing to the nearest 0.01g on a Mettler PC 400 electronic top pan balance.

For all intermediate weighings, fish were bulk weighed, without anaesthesia, every seven days. All fish in each tank were netted, using a fine mesh handnet. Excess water was then removed from the fish by blotting the net on a soft paper towel. Fish were then transferred to a tared, water-filled, container and weighed collectively to the nearest 0.01g. The weekly tank weights of fish were used to calculate the daily food ration for the following week.

2.2.4. Diet Formulation

Purified and semi-purified diets were formulated to contain 40% protein,

10% lipid and 25-30% nitrogen free extractives (NFE). These levels were based on the nutrient requirements for carp fry and fingerlings as summarized in Table 2.2. Casein, Fish meal, Mustard oilcakes, Linseed and Sesame meal were used as protein sources (detailed specifications shown in respective experiments). The diets were formulated on a dry weight basis, allowances being made for the water content of the raw ingredients during weighing. Both cod liver and soybean oils were added to the diets to achieve the desired final lipid level in the diet taking into consideration the lipid supplied incidentally by the respective plant and animal protein sources used. A mineral mixture (Table 2.3) developed by the Institute of Aquaculture was added to obviate any possible mineral deficiency in the diets. The quantity of crude fibre in the diets was kept at the desired level by the addition of α-cellulose. Vitamin premix (also developed by the Institute of Aquaculture) and binder (Sodium carboxymethyl cellulose, high viscosity) were each added at a rate of 2%. 0.5% chromium oxide was added as an inert indicator for the nutrient digestibility studies. Dextrin was added as a source of dietary carbohydrate.

2.2.5. Diet Preparation

Diets for all the experiments, except Experiment 5.2, were prepared by wet extrusion. All the dietary ingredients were finely ground and sieved through a 790μm sieve to obtain a homogenous mixture. The dry ingredients were then weighed out according to the formulation, placed in the bowl of a Hobart A200 Industrial Food Mixer (Hobart Co Ltd, London, England) and mixed until uniformly blended. To this mixture

TABLE 2.2

A summary of nutrient requirement of carp fry and fingerlings

A. PROTEIN

Stage of life/weight	% Requirement	Source	Water temp. °C	Author
Young carp (5.8g)	38	Casein	23	Ogino & Saito (1970)
Carp (4-7g)	38.4	Fish meal corn, wheat gluten	25	Sin (1973)
Fry and fingerling	43 - 47	Recommended level		National Research Council (NRC, 1983)

B. LIPID

Stage of life/weight	% Inclusion in the diet	Source	Water temp. °C	Author
Fry (0.1-0.5g)	5 - 15 10 - 15	Soybean oil Recommended level for commercial carp diet	24	Bryant (1980) NRC (1983)

C. CARBOHYDRATE

Stage of life	% Inclusion in the diet	Source	Effect	Author
Fry and fingerling	26 30	Dextrin Dextrin	Recommended level Maximum dietary level that did not reduce growth	Sen et al., (1978) Furuichi & Yone (1980)

TABLE 2.3

Composition of mineral premixes used in experimental diets[1]

MINERALS	CHEMICAL FORMULA	g/Kg
Calcium orthophosphate	$CaHPO_4,2H_2O$	727.7775
Magnesium sulphate	$MgSO_4,7H_2O$	127.5000
Sodium chloride	NaCl	60.0000
Potassium chlcride	KCl	50.0000
Iron sulphate	$FeSO_4,7H_2O$	25.0000
Zinc sulphate	$ZnSO_4,4H_2O$	5.5000
Manganese sulphate	$MnSO_4,4H_2O$	2.5375
Copper sulphate	$CuSO_4,5H_2O$	0.7850
Cobalt sulphate	$CoSO_4,7H_2O$	0.4775
Calcium iodate	$CaIO_3,6H_2O$	0.2950
Chromic chloride	$CrCl_3,6H_2O$	0.1275

[1]According to Jauncey and Ross (1982)

TABLE 2.4

Composition of the vitamin premix used in experimental diets[1]

VITAMIN	g/kg of Premix*
Thiamine (B_1)	2.50
Riboflavin (B_2)	2.50
Pyridoxine (B_6)	2.00
Pantothenic acid	5.00
Inositol	100.00
Biotin	0.30
Folic acid	0.75
Para aminobenzoic acid	2.50
Choline	200.00
Nicaine (Nicotinic acid, B_3)	10.00
Cyanocobalamin (B_{12})	0.005
Retinol palmitate (A)	100,000 IU
α-tocopherol acetate (E)	20.10
Ascorbic acid (C)	50.00
Menadione (K)	2.00
Cholecalciferol (D_3)	500,000 IU

[1]According to Jauncey and Ross (1982)

*This mixture was made up to 1kg with α-cellulose

were added weighed quantities of cod liver oil and soybean oil. Blending continued for about 10 minutes. The requisite amount of warm water (usually 20%-30%) was added slowly to the diet with continuous stirring until a stiff dough was obtained. The moist diet was extruded through a 3mm die. The resulting strands were then air dried at 35°-40°C using an electric fan convector heater. After drying pellets were ground and sieved to appropriate particle sizes. Samples of diet were then analysed for proximate composition and amino acids (Section 2.3.1 and 2.4.1). Diets were stored in polythene bags at -20°C in a deep freeze until required.

2.3.1. Methods of Proximate Analysis

Proximate analysis of diets, dietary ingredients, fish and faeces were carried out using the following procedures.

Moisture - Moisture content was determined by air-drying the samples in an oven at 105°C for 24 hours.

Crude Protein - Crude protein was determined by the micro-kjeldhal method for determining nitrogen (AOAC Methods, 1980) and applying the empirical factor of 6.25 to the results to convert total nitrogen to total protein. This assumes that protein contains 16% nitrogen.

Crude Lipid - Crude lipid content was determined by the freon (Trichlor ofluoro methane 99%) extraction method (Korn and Macedo,1973). This method was selected because of its reliability coupled with speed and

accuracy and the small quantities of samples required for analysis.

Ash - Ash content was determined by igniting the samples in a muffle furnace overnight at a temperature of 450°C (AOAC, 1980).

Crude Fibre - Crude fibre was analysed after AOAC 1980 (loss on ignition of dried lipid-free residues remaining after digestion with 1.25% H_2SO_4 and 1.25% NaOH).

Nitrogen Free Extractives (NFE) - NFE was estimated by subtracting the total of moisture, crude protein, crude lipid, ash, and crude fibre from 100.

2.3.2. Chromic Oxide Analysis

50-100mg triplicate portions of moisture free diets and faeces were analysed for chromic oxide content after the method of Furukawa and Tsukahara (1966). The chromic oxide contents were expressed as a percentage of the samples.

2.4.1. Amino Acid Analysis

The amino acid contents of the dietary protein sources, diets, faeces and carcass samples were analysed according to the procedure described below.

Sample Preparation - Samples for amino acid analysis were hydrolysed with 5.7 NHCl for 24 hours at 110°C in vacuo, following the procedures given in the LKB 4151 Alpha-Plus Instruction manual (1983).

Analysis - All the analyses were carried out using an LKB 4151 Alpha-Plus Amino Acid Analyser (LKB Bichrom Ltd, Cambridge). These analyses were based on the following principles. The prepared sample was loaded on to a column of cation-exchange resin. The amino acids were sequentially eluted by buffers of varying pH and ionic strength. In a high temperature reaction coil ninhydrin was reacted with the column eluent to form coloured compounds. The colour intensity being directly proportional to the quantity of an amino acid present in the sample. The amount of each coloured compound is determined by a photometer measuring the amount of light absorbed at 570nm. Amino acids were identified by comparison of peak retention times to a known standard and were quantified by comparison of peak areas to the same standard mixture.

2.4.2. Available Lysine Analysis

The concept of "available" as distinct from total amino acid present in a protein is used to differentiate between amino acids modified or damaged in some way with consequent loss of nutritive value, and those which remain nutritionally available to metabolic process (Roach et al., 1967). The amino acid lysine has received major attention in this respect since it is very susceptible to heat damage and is often a limiting essential amino acid in oilseed meals and cereals used in compound feedstuff.

The available lysine levels in the protein sources used were analysed following the method of Roach et al. (1967). Available lysine was calculated by measurement of the total lysine in an acid hydrolysate and also of lysine remaining in solution after a separate hydrolysis of the protein treated with 1-fluoro-2,4-dinitrobenzene (FDNB). The difference between these two values represents the lysine in the protein which has free ε-amino groups and is interpreted as "available" lysine.

2.5.1. Energy Determination

The energy contents of the diet and faeces were analysed using an Automatic Adiabatic Bomb Calorimeter (Gallenkamp & Co Ltd, Loughborough, England).

2.6.1. Mineral Analysis

Samples (1.0g) of diet and carcass were digested (24 hours) with concentrated nitric acid (15ml) at 110°C until a clear solution was obtained. On cooling the solution was filtered through ashless filter paper and made up to a definite volume with de-ionised water. Analysis of Ca, K, Na, Mg, Fe, Zn, Mn, Cu was carried out using a Perkin Elmer 2280 Atomic Absorption Spectrophotometer according to the manufacturer's specification. Phosphorous was measured by the method outlined by Stirling (1985). The principle of the method is that phosphorous in the sample is converted to soluble inorganic phosphorous by digestion with

nitric acid and perchloric acid. This phosphorous reacts with molybdate to form molybdophosphoric acid which is then reduced to the intensely coloured molybdenum blue complex and determined spectrophotometrically.

2.7.1. Anti-nutritional Factor Analysis

(a) **Allyl Isothiocyanate** - Allyl isothiocyanate in mustard oilcake was determined by the widely used argentimetric method of Wetter (1955).

(b) **Phytic Acid** - Phytic acid in sesame and linseed meal was analysed by the method proposed by Wheeler and Ferrel (1971). Extraction of the samples with 3% TCA is followed by quantitation of the Fe^{3+} required to precipitate the extracted phytic acid.

(c) **Hydrocyanic Acid (HCN)** - The concentration of hydrocyanic acid in linseed meal was determined according to the AOAC (1980) method of analysis for content of cyanogenetic glucosides in feedstuffs.

(d) **Tannins** - Tannin in mustard oilcake was determined by the method for tannins in cloves and allspice (AOAC, 1980).

2.8.1. Analysis of Experimental Data

Data collected during the growth trial and subsequent analysis of diets,

carcasses and faeces were used for the determination of various nutritional parameters.

(i) Growth

(a) Percentage weight gain

$$\% \text{ weight gain} = \frac{\text{mean final fish wt} - \text{mean initial fish wt}}{\text{mean initial fish weight}} \times 100$$

(b) Specific Growth Rate (SGR)

The SGR is the instantaneous change in weight of fish calculated as the percentage increase in body weight per day over any given time interval as follows:

$$\text{SGR (\% day)} = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1} \times 100 \quad \text{(after Brown, 1957)}$$

Where,

W_1 = the initial live body weight (g) at time T_1 (day)

W_2 = the final live body weight (g) at time T_2 (day)

(ii) Food Conversion Ratio (FCR)

The food conversion ratio is defined as the amount of dry food fed per unit live weight gain. It is calculated as

$$\text{FCR} = \frac{\text{Food fed (dry weight)}}{\text{Live Weight gain}}$$

For calculation of FCR, the dry weight of the food was obtained by using a correction for the analysed moisture content of the

diet. FCR is a measure of the degree of gross utilization of food for growth.

(iii) **Protein Utilization**

(a) Protein Efficiency Ratio (PER)

PER is defined as the gain in weight of fish per gram of crude protein fed. This was calculated as

$$PER = \frac{\text{Live weight gain(g)}}{\text{Crude protein fed (g)}}$$

PER gives an indication of the efficiency of utilization of dietary protein. PER does not allow for the fact that weight gain may also be due to changes in carcass lipid and moisture content rather than protein.

(b) Apparent Net Protein Utilization (ANPU)

Net protein utilization (NPU) is an improved measure of protein utilization. NPU expresses the percentage of ingested protein that is retained by deposition in the carcass. It is usually determined by the carcass analysis method of Miller and Bender (1955). Since no correction was made for the endogenous nitrogen losses during these experiments, results expressed as Apparent NPU given by

$$ANPU\ (\%) = \frac{Nb - Na}{Ni} \times 100$$

Where,

Na = the body nitrogen at the start of the test

Nb = the body nitrogen at the end of the test

Ni = the amount of nitrogen ingested

Due to practical constraints in experiments with fish it was not possible to ensure that all food presented was ingested nor was it possible to collect uneaten food from the experimental tanks. Therefore for calculation of FCR, PER and ANPU the amount of food offered was used without correction being made for any wastage.

(iv) **Digestibility Determination**

The use of inert indicators, which pass unaffected by digestion through the alimentary tract, has provided a convenient method of measuring digestibility wihout the need to collect the faeces quantitatively. This method has been successfully applied to fish using chromic III oxide as the indicator (Furukawa and Tsukahara, 1966). Dry matter (DM) digestibility is calculated according to the formula (Windell et al., 1978b):

$$\% \text{ DM} = 100 - 100\left[\frac{\% \text{ } Cr_2O_3 \text{ in feed}}{\% \text{ } Cr_2O_3 \text{ in faeces}}\right]$$

Estimates of apparent nutrient (protein, lipid, ash, energy) digestibility were derived from the following equation (Maynard and Loosli, 1969),

$$\text{Apparent nutrient digestibility (\%)} = 100 - \left(100 \times \frac{\%\ Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in feed}}\right)$$

2.9.1. Statistical Analysis

Statistical analyses in this study were performed using the Minitab Statistical Package (Ryan, Joiner and Ryan, 1976) on the Stirling University's mainframe computer (VAX A). Comparison between treatment means was carried out by analysis of variance followed by Duncan's Multiple Range Test (Duncan, 1955) to test the significance of variation between the means. Standard errors (±SE) of treatment means were calculated from the residual mean squares in the analysis of variance.

CHAPTER 3 : EXPERIMENT 1

Substitution of fish meal by locally available Bangladeshi oilseed by-products in the diet of common carp (Cyrpinus carpio L)

3.1 INTRODUCTION

In fish culture fish meal is generally regarded as an essential ingredient in complete fish feeds. This dependence of intensive and semi-intensive aquaculture on fish meal presents a severe constraint to the development and intensification of this industry in many regions. Fish meal supply in the world market is not constant and the price is ever increasing. For both economic and practical reasons fish feeds should use locally available protein sources, preferably those unsuitable for direct human consumption.

In recent years intensification of carp fry and fingerling production in Bangladesh and the proposed expansion of aquaculture for the next 20 years (FAO/UNDP, 1985) has generated the need for development of suitable feeds. Fish meal is widely used as a major dietary protein source in fish feed throughout the world. However, in Bangladesh the use of fish meal at high levels in fry and fingerling feeds is not feasible because of its high price and limited supply. Therefore, it is necessary to look for cheap, locally available alternative protein sources to develop suitable feeds for carp.

Many attempts have been made to replace fish meal, partially or completely, by various plant protein sources. Considerable research effort has been directed at the use of single cell proteins (SCP) such as yeast (Candida lipolytica i.e. Toprina), bacterial SCP (Methylophilus methylotrophus i.e. pruteen) and algal SCP as the sole or principal sources of dietary protein in fish feeds (Tiews et al., 1979; Kaushik and Luquet, 1980; Tacon et al., 1983a; Appler and Jauncey, 1983). The

studies of Appler and Jauncey (1983) and Appler (1985) with tilapia and of Hepher et al. (1979) and Meske and Pfeffer (1978) with common carp indicate that certain dried algal meals show promise as a partial dietary replacement for fish meal.

Attempts to replace fish meal with commercially processed soybean meals have met with variable success. Dabrowski and Kozak (1979) reported poor growth response and food utilization of carp fry with diets containing 40% soybean meal compared to a fish meal based control diet. On the contrary, Viola et al. (1982) successfully replaced 40% fish meal with soybean meal in carp diets and observed similar growth performance and protein efficiency to those of fish fed a fish meal diet. Jackson et al. (1982) and Shiau et al. (1987) also reported that at 25% and 30% inclusion level respectively, soybean meal supported comparable growth to fish meal based control diets in tilapia.

Dorsa et al. (1982) observed that growth in channel catfish (Ictalurus punctatus) was inhibited when fish were fed diets with more than 17.40% cottonseed meal or with 0.09% or more gossypol. Dickson (1981) and Jackson et al. (1982) indicated that cottonseed meal is a promising source of protein in tilapia diets even at 100% level of inclusion. In contrast, Robinson et al. (1984) reported that glanded and glandless cottonseed products were not as high in nutritive value for tilapia as for soybean and peanut meals.

Dabrowski and Kozlowska (1981) fed rapeseed meal diets containing high (4.93 mg/g) and low (0.15 mg/g) levels of glucosinolates to carp and

found that the former caused a marked depression in growth. Jackson et al. (1982) reported that rapeseed meal (low-glucosinolate) promoted reasonable growth in S. mossambicus when it provided 50% of total dietary protein. However, when the level of inclusion was increased to 75% there was a significant reduction in growth rate.

Information regarding the efficiency of mustard, linseed and sesame meals as alternative protein sources in fish feed is scanty. Nutritional evaluation of mustard oilcake has been carried out by several researchers (Laksmannan et al., 1967; Chakrabarty et al., 1973; Chowdhary et al., 1978; Capper et al., 1982). All studies, except that of Capper et al. (1982), have been conducted in earthern rearing ponds where natural food was available to fish. Some of these dietary ingredients may therefore have acted as pond fertilizers (providing the nutrients for plankton production) rather than being used directly by the fish. Therefore, from the observed results, it is impossible to evaluate the contribution of this ingredient to fish production alone.

Like other plant oilseed meals mustard oilcake is reported to contain a variety of endogenous anti-nutritional factors such as goitrogenic glucosinolates (allyl isothiocyanate) (Mustakus et al., 1962; Shah et al., 1977; Maheswari et al., 1981;) and tannins (Capper et al., 1982). Similarly linseed and sesame meals contain phytic acid and cyanogenic glucosides (HCN) (Erdman, 1979; Cheryan, 1980 and Madhusudhan and Singh, 1983).

In view of the absence of information regarding the nutritive value of

mustard oilcake, linseed and sesame meals to fish, a preliminary eight week feeding trial was conducted with carp fry employing these protein sources as fish meal substitutes. The aim of this experiment was to determine the maximum inclusion levels for different plant protein sources and to identify the limitations of their use arising from palatibility or apparent toxicity factors.

3.2 MATERIALS AND METHODS

The source of experimental animals and their quarantine procedures are described in Section 2.2.1.

3.2.1. Experimental System

The experimental systems described in Section 2.1.1 and Section 2.1.2 were used for growth trial and faeces collection respectively.

3.2.2. Analytical Techniques

The proximate composition analysis of the ingredients, prepared diets, faeces and carcass samples were carried out following the methods described in Section 2.3.1. The amino acid content of the ingredients and diets were analysed according to the method described in Section 2.4.1. The amino acid tryptophan could not be analysed due to its destruction during acid hydrolysis. Therefore, tryptophan values for all ingredients were obtained from NRC (1983) except for that of mustard oilcake, which was obtained from Capper et al. (1982). Calcium, phos-

phorous and available lysine contents of the ingredients and diets were analysed by the methods described in Section 2.6.1 and 2.4.2 respectively. The anti-nutritional factors in plant protein sources were analysed according to the methods described in Section 2.7.1. The chromic oxide contents of the diets and faeces was determined by the method described in Section 2.3.2. The energy content of the diets and faeces was determined as described in Section 2.5.1.

3.2.3. Diet Formulation and Preparation

Eight semi-purified isonitrogenous diets were formulated to evaluate the following plant protein sources locally available in Bangladesh:

(1) Indian mustard (Brassica juncea)

- expeller cake; origin Bangladesh

(2) Linseed (Linum usitatissimum)

- expeller cake; origin Bangladesh

(3) Sesame (Sesamum indicum)

- expeller type, origin India which is likely to be similar to sesame meal available in Bangladesh.

Mustard oilcake and linseed meal were imported directly from Bangladesh for this study. Because of delays in supply from Bangladesh, sesame meal was obtained from Salamon and Seabers Ltd, Old Street, London.

These protein sources and herring meal were analysed for proximate composition and amino acid content prior to diet formulation. Results of the proximate composition and amino acid analysis (for methods see Section 3.2.2) are presented in Table 3.1 and 3.2 respectively. Calcium, phosphorous, available lysine and the anti-nutritional factors in the feed ingredients were also analysed and are presented in Table 3.1.

Eight diets were formulated to contain 40% protein, 10% lipid, 8% crude fibre and about 25% nitrogen free extractives (NFE). The level of protein used in the present trial was based on the nutritional requirement of carp fry and fingerlings as shown in Table. 2.2. However, this protein level is lower than the optimum dietary protein level recommended by National Research Council (NRC, 1983) for carp fry and fingerlings but higher than reported by Ogino and Saito (1970) and Sin (1973). Carboxymethyl cellulose and dextrin were used as binder and carbohydrate source respectively. The compositions of the vitamin and mineral premixes used are shown in Section 2.2.4.

Mustard oilcake and linseed meals were tested at two inclusion levels (25% and 50%) and sesame meal was tested at three inclusion levels (25%, 50% and 75% of total protein). The levels of inclusion of test protein sources in the experimental diets are shown in Table 3.3. One hundred percent replacement of fish meal was not possible as none of the ingredients tested contained sufficient protein to supply 40% in a balanced formulated diet.

The control diet was prepared with fish meal as the sole source of

TABLE 3.1

Proximate composition, Ca, P, anti-nutritional factors and available lysine content of the dietary ingredients used in Experiment 1 and 2 (% dry matter basis)

Components	Ingredients			
	Fish meal	Mustard oilcake	Linseed meal	Sesame meal
Dry matter	92.75	87.00	91.83	91.83
Crude protein	76.40	32.74	33.73	43.09
Crude lipid	9.09	12.25	6.32	7.67
Ash	11.73	10.36	7.98	13.65
Crude fibre	0.51	11.47	10.04	6.70
NFE[1]	2.27	33.18	41.93	28.89
Ca	2.35	0.85	0.69	2.24
P	2.10	1.62	0.83	1.38
Allyl isothiocyanate	-	0.54	-	-
Phytic acid	-	-	2.64	2.86
Hydrocyanic acid (HCN)	-	-	0.043	-
Tannins	-	1.87	-	-
Available lysine	4.57	1.37	0.80	1.00
(% of total lysine)	(91.40)	(83.03)	(81.63)	(80.00)

[1]Nitrogen free extractives calculated as

100 - (Moisture+Crude protein+Crude lipid+Ash+Crude fibre)

TABLE 3.2

Essential amino acid (EAA) composition (% dry matter) of the dietary protein sources used in Experiment 1 and 2

Amino acids	Fish meal	Mustard oilcake	Linseed meal	Sesame meal
Arginine	3.65 (4.78)*	1.90 (5.80)	2.23 (6.61)	4.76 (11.04)
Histidine	1.94 (2.54)	0.88 (2.68)	0.59 (1.75)	0.99 (2.29)
Isoleucine	2.65 (3.47)	1.28 (3.91)	1.08 (3.20)	1.30 (3.01)
Leucine	5.09 (6.66)	2.31 (7.05)	1.61 (4.77)	2.81 (6.52)
Lysine	5.00 (6.54)	1.65 (5.04)	0.98 (2.90)	1.25 (2.90)
Methionine	1.95 (2.55)	0.69 (2.10)	0.26 (0.77)	0.76 (1.76)
Cystine	0.49 (0.64)	0.44 (1.34)	0.12 (0.36)	0.73 (1.69)
Phenylalanine	2.54 (3.32)	1.48 (4.52)	1.27 (3.76)	2.03 (4.71)
Tyrosine	2.23 (2.92)	1.19 (3.63)	0.73 (2.16)	1.70 (3.95)
Threonine	3.23 (4.23)	1.80 (4.50)	1.17 (3.47)	1.72 (3.99)
Tryptophan**	0.84 (1.07)	0.52 (1.19)	0.55 (1.63)	0.76 (1.55)
Valine	3.16 (4.14)	1.54 (4.70)	1.23 (3.65)	1.94 (4.50)

* Figures within the parenthesis indicate the EAA contents expressed as percentage of protein

** Tryptophan values were obtained from NRC (1983) except that of mustard oilcake which was obtained from Capper et al. (1982)

TABLE 3.3

Level of inclusion of different test proteins (as % total crude protein) in the diets used in Experiment 1

Test protein sources	DIET NO.							
	1 (Control)	2	3	4	5	6	7	8
Fish meal	100	75	50	75	50	75	50	25
Mustard oilcake	-	25	50	-	-	-	-	-
Linseed meal	-	-	-	25	50	-	-	-
Sesame meal	-	-	-	-	-	25	50	75

TABLE 3.4

Formulation of the experimental diets used in Experiment 1

Ingredients	DIET NO.							
	1 (Control)	2	3	4	5	6	7	8
Mustard oilcake	-	30.50	61.00	-	-	-	-	-
Linseed meal	-	-	-	29.75	59.25	-	-	-
Sesame meal	-	-	-	-	-	23.25	46.50	69.75
Fish meal	52.50	39.25	26.25	39.25	26.25	39.25	26.15	13.00
Cod liver oil	0.23	1.43	1.14	1.43	2.61	1.43	2.62	3.47
Soybean oil	5.00	1.26	-	3.12	1.26	3.22	1.43	-
Mineral premix[1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin premix[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Binder (CMC)[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Dextrin	25.77	15.06	2.61	12.95	0.63	18.35	10.80	2.78
α-cellulose	8.00	4.00	0.50	5.00	1.50	6.00	4.00	2.50
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1] For composition of the mineral and vitamin premixes see Tables 2.3 and 2.4 respectively

[2] Sodium carboxymethyl cellulose (high viscosity)

TABLE 3.5

Analysed composition of the experimental diets used in Experiment 1
(% dry matter basis unless otherwise stated)

Components	DIET NO.							
	1 (Control)	2	3	4	5	6	7	8
Dry matter	94.54	94.64	94.22	95.20	95.20	95.14	95.27	95.05
Crude protein	40.62	40.01	40.26	40.33	39.82	40.24	39.50	40.49
Crude lipid	9.46	9.58	10.32	10.17	10.28	9.50	9.49	9.38
Crude fibre	8.24	7.77	7.65	8.09	7.88	7.88	7.16	7.63
Ash	9.71	10.94	12.36	10.50	10.91	11.29	13.07	14.09
NFE[1]	31.97	31.70	29.41	30.91	31.11	31.09	30.78	28.41
Ca	1.70	1.66	1.64	1.58	1.61	1.68	1.78	1.94
P	1.59	1.63	1.67	1.61	1.56	1.65	1.69	1.75
Total energy (Kcal/g)	4.48	4.42	4.40	4.46	4.53	4.42	4.41	4.33
PE Ratio[2]	90.67	90.52	91.50	90.43	87.90	91.04	89.57	93.51
Available lysine	2.29	2.15	1.72	1.71	1.65	1.76	1.51	1.29
(% of total lysine)	(90.51)	(87.39)	(84.31)	(86.36)	(84.18)	(86.27)	(82.51)	(79.62)
Allyl isothiocyanate	-	0.16	0.33	-	-	-	-	-
Phytic acid	-	-	-	0.78	1.56	0.66	1.33	1.99
HCN[3]	-	-	-	0.013	0.026	-	-	-
Tannins[3]	-	0.57	1.14	-	-	-	-	-
Chromic oxide	0.49	0.50	0.51	0.52	0.52	0.48	0.49	0.51

[1] Nitrogen free extractives calculated as
100 - (Moisture+Crude protein+Crude lipid+Ash+Crude fibre)

[2] Protein to energy ratio in mg protein/KCal of total energy

[3] Levels of anti-nutritional factors were estimated from their levels in the dietary protein sources as described earlier (Table 3.1)

TABLE 3.6

Analysed essential amino acid (EAA) composition of the experimental diets used in Experiment 1 (% dry matter basis)

Amino Acids	DIET NO.								Requirement for carp[1]
	1 (Control)	2	3	4	5	6	7	8	
Arginine	2.06	2.01	2.13	2.04	2.81	2.72	3.01	3.42	1.52
Histidine	0.74	0.95	0.83	0.70	0.71	0.83	0.67	0.66	0.56
Isoleucine	1.41	1.32	1.26	1.24	1.27	1.60	1.31	1.21	0.92
Leucine	2.53	2.69	2.34	2.13	2.30	2.80	2.40	2.20	1.64
Lysine	2.53	2.46	2.04 (96.2)[2]	1.98 (93.4)	1.96 (92.5)	2.04 (96.2)	1.83 (86.3)	1.62 (76.4)	2.12
Methionine	0.92	0.81	0.50 (78.1)	0.60 (93.7)	0.53 (82.8)	1.08	0.57 (89.1)	0.56 (87.5)	0.64
Cystine	0.23	0.22	0.22	0.21	0.39	0.30	0.28	0.42	-
Phenyl alanine	1.20	1.50	1.10 (94.8)	1.13 (97.4)	1.34	1.53	1.62	1.46	1.16
Tyrosine	0.84	1.13	0.82	0.64	0.86	1.11	0.94	0.84	-
Threonine	1.43	1.46	1.28 (96.9)	1.20 (90.9)	1.27 (96.2)	1.77	1.26 (95.5)	1.17 (88.6)	1.32
Tryptophan[3]	0.44	0.49	0.53	0.49	0.48	0.50	0.57	0.64	0.24
Valine	1.62	1.66	1.68	1.52	1.66	2.00	1.73	1.76	1.16

[1] Data for EAA requirement of carp from Ogino (1980)

[2] Figures within parenthesis indicate chemical scores of deficient EAAs

[3] Estimated from the levels in the dietary protein sources as described earlier (Table 3.2)

TABLE 3.7

A summary of the methodology used to evaluate the protein sources locally available in Bangladesh as substitute for fish meal in carp fry diet

Fish	C. carpio, average initial weight 1.06±.04g
Duration of experiment	8 weeks
Treatments	Evaluation of mustard oilcake, linseed and sesame meal as dietary protein substitute for common carp
No. of treatments	8
Replication	4/treatment
Water temperature	27° ± 1°C
Stocking density	15/tank
Water flow rate	1 litre/min/tank
Carcass sampling	Initial sample - 20 fish at the start of experiment. Final sample - 20 fish per treatment (5 from each replicate)
Faecal collection	Collected twice daily for two weeks, dried at 60°C and used for digestibility study
Physico-chemical characteristics monitored	Temperature, pH, dissolved oxygen and total ammonia

protein. Each diet was formulated to contain 0.5% chromic oxide to study the nutrient digestibility of the experimental diets. The formulations of the experimental diets are presented in Table 3.4. All the prepared diets were analysed for proximate composition, Ca, P, and available lysine as presented in Table 3.5. The amino acid contents of the diets were analysed and are presented in Table 3.6.

Detailed procedures for the diet formulation and preparation were similar to those described in Section 2.2.4 and 2.2.5 respectively.

3.2.4. General Experimental Procedure

There were four replicates for each treatment with 15 fish in each replicate. Fry were acclimated to the experimental system for seven days before the start of the experiment. During acclimation, fish were fed trout pellet (Ewos Baker's Omega No.3, 49% protein). Weighing and acclimation procedures were as described in Section 2.2.3. Weighing of the fish during the experiment was carried out every seven days. The experiment was conducted for a period of eight weeks. A summary of the methodology used in the present investigation is given in Table 3.7.

3.2.5. Feeding Rates

The fish were fed three times per day between 09.00 hours and 17.00 hour, at four hourly intervals. Fish in each tank were fed 10% of their total biomass per day. Each of the three daily feeds was administered over a

period of 15 minutes to ensure consumption of the whole ration. After week 4 it was observed that fish were unwilling to consume the whole ration. The feeding rate was thus reduced to 6% of the body weight per day at this point. As the moisture contents of all diets were low and similar, no correction of feeding rates was made for moisture content. A record of the amount of food fed was kept for subsequent calculation of food conversion and protein utilization. The quantity of food fed per day was adjusted after each weekly weighing and fed for the subsequent week. Any mortality during the experimental period was recorded.

3.2.6. Faeces Collection

At the beginning of the seventh week, the fish were transferred to the faecal collection system (see Section 2.1.2 for details). Faeces were collected twice daily - morning and evening - for two weeks. The faeces deposited in each tank bottom were collected separately in small beakers by opening the valve at the bottom of each tank. Collected faeces were dried in an oven at 60°C. The faecal samples of each tank were pooled to represent respective treatments and stored in air-tight containers for subsequent chemical analysis.

3.2.7. Histological Techniques

At the start of Experiment 10 fish were fixed in 10% buffered formalin as an initial sample for histopathological study. Similarly, at the end of the experiment 12 fish from each treatment, including control, were fixed

in 10% buffered formalin, processed by routine methods, embedded in paraffin wax and sectioned by microtone set at 5 micron. The tissue sections were stained with haemotoxylin and eosin and examined under a light microscope to assess any histopathological changes in the gills, thyroid, liver, muscle, kidney and intestine.

3.2.8. Analysis of Experimental Data

Analysis of the results was conducted as described in Section 2.8.1. Statistical analyses were performed as described in Section 2.9.1.

3.3 RESULTS

3.3.1. Analysed Composition of the Experimental Diets

The protein level in all experimental diets was very similar (Table 3.5). This also applies to the levels of crude lipid and crude fibre. Ash contents in Diets 7 and 8 were higher than for the rest of the diets. Energy contents varied little between the diets (from 4.40 to 4.53 Kcal/g). The available lysine content varied from 2.29% in the control to 1.29% in Diet 8 (75% sesame). From Table 3.6 it is seen that Diets 3, 4, 5, 6, 7 and 8 are deficient in one or more essential amino acids mainly in lysine, methionine and phenylalanine. All experimental diets, except the control, contained various types and levels of anti-nutritional factors as shown in Table 3.5.

3.3.2. Acceptability of Diets

The acceptability of different experimental diets was judged by a subjective behavioural assessment of the feeding responses. All fish acclimated to the experimental diets within 2-3 days of the start of feeding. However the acceptability of all diets was not similar. The Control diet and the diets containing 25% plant protein (mustard and linseed) were generally more acceptable. The diets with higher levels of plant protein inclusion (50%-75% of total protein) were less readily accepted. A summary of these observations is shown in Table 3.8.

3.3.3. Mortality

The only mortalities were in fish fed Diet 8 (75% sesame). The final total mortality was 18.33% with fish dying from week 6 onwards.

3.3.4. General Health and Histological Examination

Fish fed Diet 8 (75% protein from sesame) showed poor health and some malformations (Plate 3.1-3.2). These physical deformities were probably caused by lowered food intake, nutritional imbalances or the presence of anti-nutritional factors in the diet. Histopathological examination of the gills, liver, muscle, thyroid, kidney and intestine of fish fed the various dietary treatments revealed no significant changes except for the diet containing the highest level of mustard oilcake (Diet 3).

TABLE 3.8

Observation on the acceptability of different diets containing plant proteins fed to carp fry

Diet No.	Inclusion of plant protein (% of total protein)	Observation on acceptability
1	Control (100% fish meal protein)	Fish were feeding actively throughout the trial; food taken and swallowed directly; all the food consumed within 15 minutes of administration; less faeces observed
2	25% Mustard	
4	25% Linseed	
3	50% Mustard	Initially fish were feeding actively but towards the end of trial acceptability was moderate; sometimes uneaten food present in tank; large amount of faeces observed
5	50% Linseed	
6	25% Sesame	
7	50% Sesame	Acceptability was poor; from week 4 onwards feeding activity deteriorated largely due to fish's poor health; frequently large amount of uneaten food accumulated
8	75% Sesame	

PLATE 3.1

Lateral view of carp fed Diet 8 (75% sesame meal protein) from Experiment 1 showing poor health.

PLATE 3.2

Fish fed the fish meal based Control diet from Experiment 1 showing normal health.

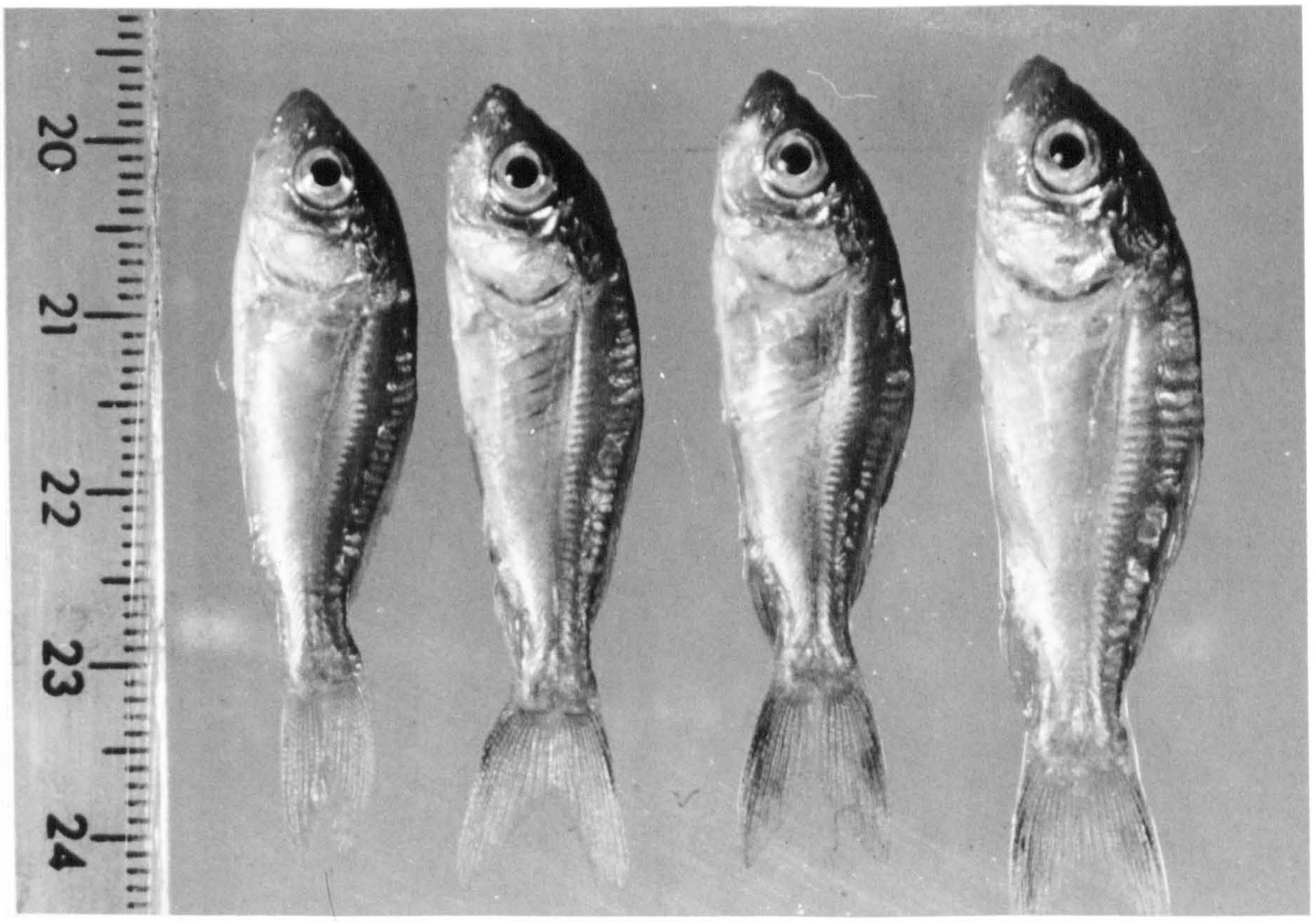


Plate 3.1

Plate 3.2

PLATE 3.3

Thyroid follicles in kidney tissues of carp fed fish meal based Control diet from Experiment 1. Note fewer number and circular shape of the follicles.

(x250)

PLATE 3.4

Thyroid follicles in kidney tissues of carp fed Diet 3 (50% mustard protein) from Experiment 1. Note the variable sizes, shapes and general packed appearance of the follicles.

(x250)

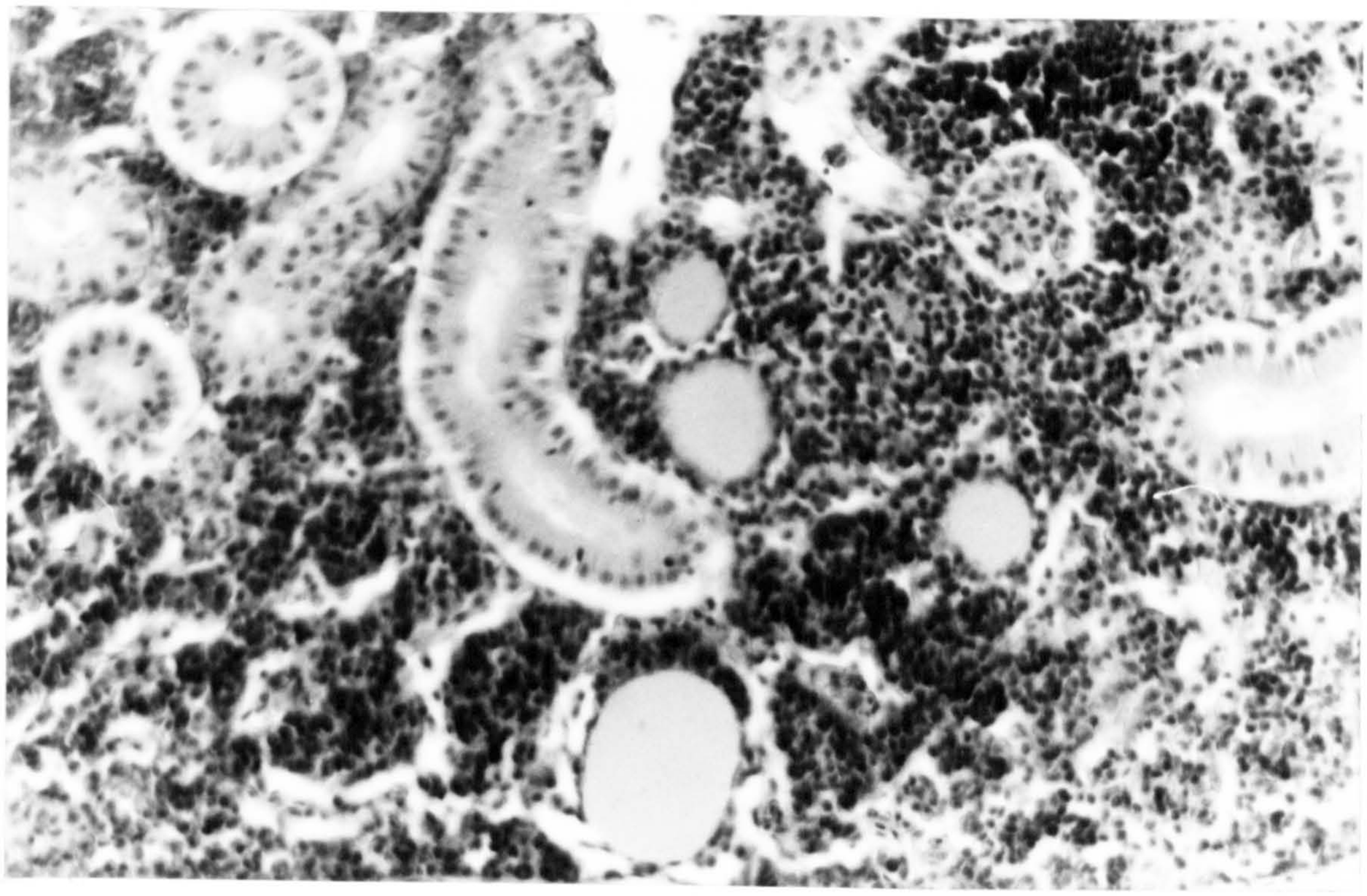


Plate 3.3

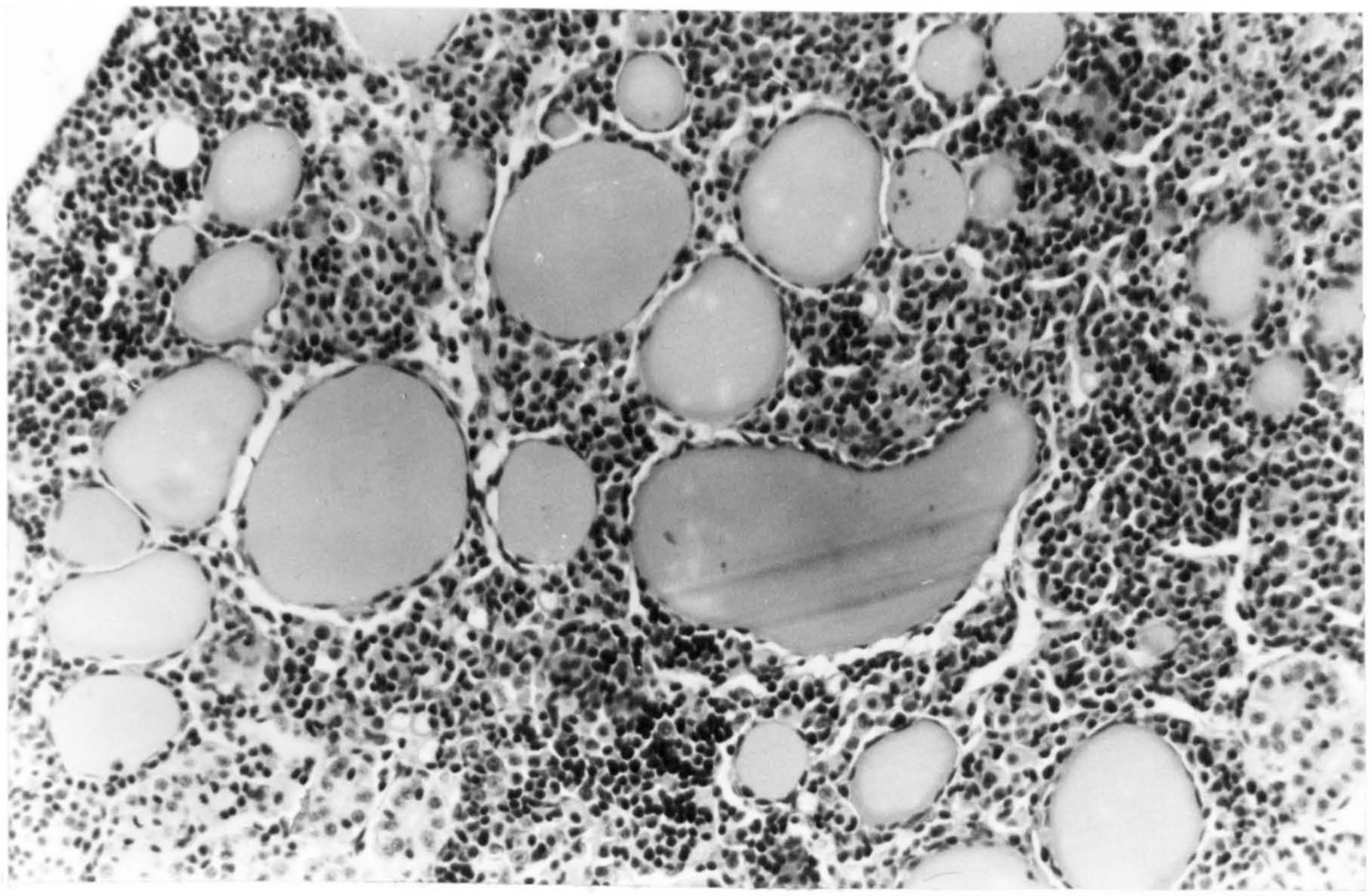


Plate 3.4

PLATE 3.5

Section of liver from carp fed Diet 3 (50% mustard protein) from Experiment 1 showing severe intracellular lipid deposition.

(x400)

PLATE 3.6

Section of liver from carp fed fish meal based Control diet from Experiment 1. Note no visible intracellular lipid deposition.

(x400)

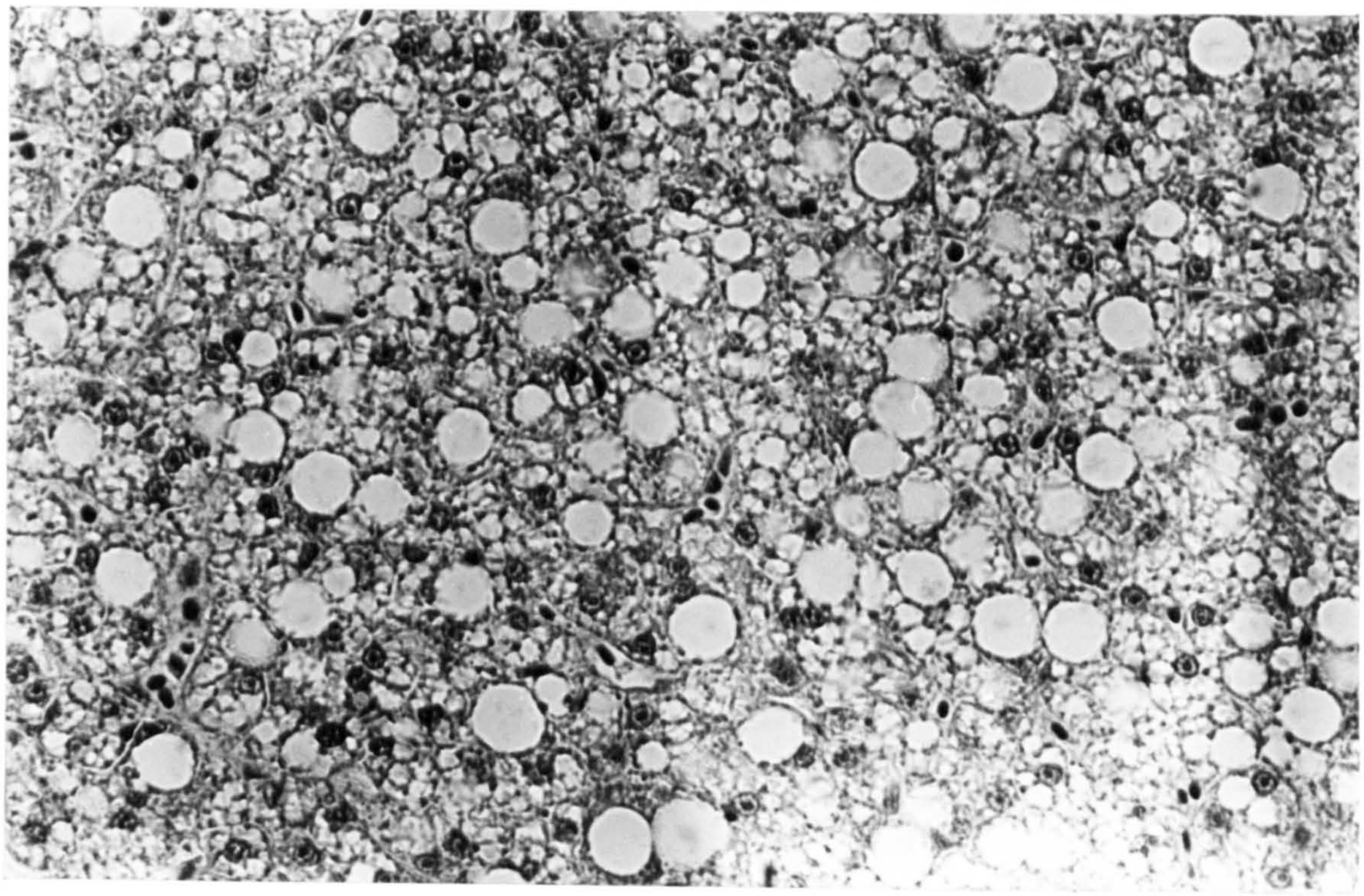


Plate 3.5

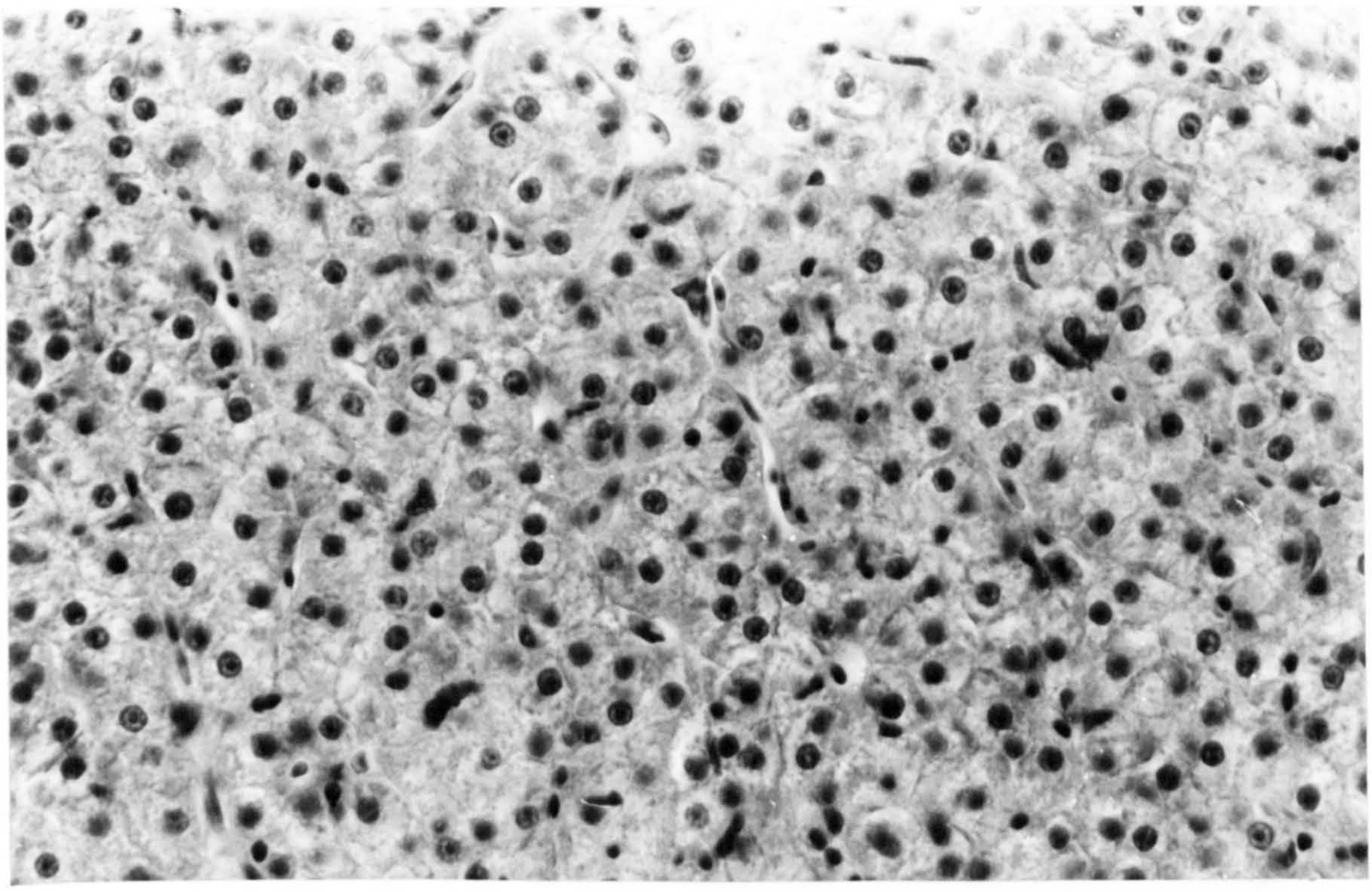


Plate 3.6

Thyroid tissue in carp is unusually distributed being located in pharyngeal and renal tissues. Kidney thyroid centres are several times more active than those in the pharyngeal region of juvenile carp (Lysak, 1979). In the present experiment thyroid tissues in all groups were generally scattered throughout the kidney tissues. Fish fed the Control diet had fewer follicles (Plate 3.3). These follicles were circular in shape with abundant pink colloid. Diet 3 (50% mustard) showed an increase in follicular epithelium (Plate 3.4). Follicles displayed a wide range of sizes and many follicles were elongated. Some of the follicles had a little or no colloid. The follicle epithelial cell height was slightly greater in fish fed Diet 3 than in the control.

Liver histology revealed that liver cells of fish fed Diet 3 (50% mustard) contained a higher level of intracellular lipid deposition compared to fine and evenly distributed granular cytoplasm noted in the hepatocyst of the groups fed Control diet (Plate 3.5 and 3.6).

3.3.5. Growth

Growth responses of carp fry fed the experimental diets are presented as initial and final mean weights, percentage weight gain and specific growth rate (SGR) in Table 3.9 and graphically in Figure 3.1. It appears that growth responses were significantly affected by both the type and inclusion level of plant protein. In general, the growth rate decreased with the increase in the level of inclusion of plant protein. The Control diet (herring meal) produced best growth response throughout the

TABLE 3.9

Growth and food utilization of common carp fry fed the experimental diets for 8 weeks

Components	Mean Values DIET NO. 1 (Control)	2	3	4	5	6	7	8	± S.E.[2]
Initial weight (g)	1.08^{a1}	1.11^{a}	1.09^{a}	1.10^{a}	1.08^{a}	1.06^{ab}	1.01^{b}	1.02^{b}	0.02
Final weight (g)	8.02^{a}	6.15^{b}	4.21^{d}	6.23^{b}	4.08^{d}	4.67^{c}	2.98^{e}	2.08^{f}	0.08
Weight gain (g)	5.94^{a}	5.04^{b}	3.12^{cd}	5.13^{b}	3.00^{d}	3.61^{c}	1.97^{e}	1.08^{f}	0.07
% weight gain	643^{a}	454^{b}	286^{d}	466^{b}	278^{d}	341^{c}	194^{e}	106^{f}	5.47
SGR (% day)	3.58^{a}	3.05^{b}	2.41^{d}	3.09^{b}	2.37^{d}	2.65^{c}	1.93^{e}	1.29^{f}	0.02
SGR as % of Control	100	85.19	67.32	86.31	66.20	74.02	53.91	36.03	-
% Mortality	-	-	-	-	-	-	-	18.33	-
FCR	2.09^{f}	2.46^{e}	3.05^{cd}	2.39^{e}	3.16^{c}	2.94^{d}	3.68^{b}	6.32^{a}	0.04
PER	1.17^{a}	1.02^{b}	0.81^{cd}	1.04^{b}	0.79^{d}	0.84^{c}	0.65^{e}	0.39^{f}	0.01
ANPU (%)	17.82^{a}	15.05^{b}	11.41^{cd}	15.51^{b}	11.06^{d}	12.25^{c}	9.11^{e}	5.41^{f}	0.34

[1] Figures in the same row having the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

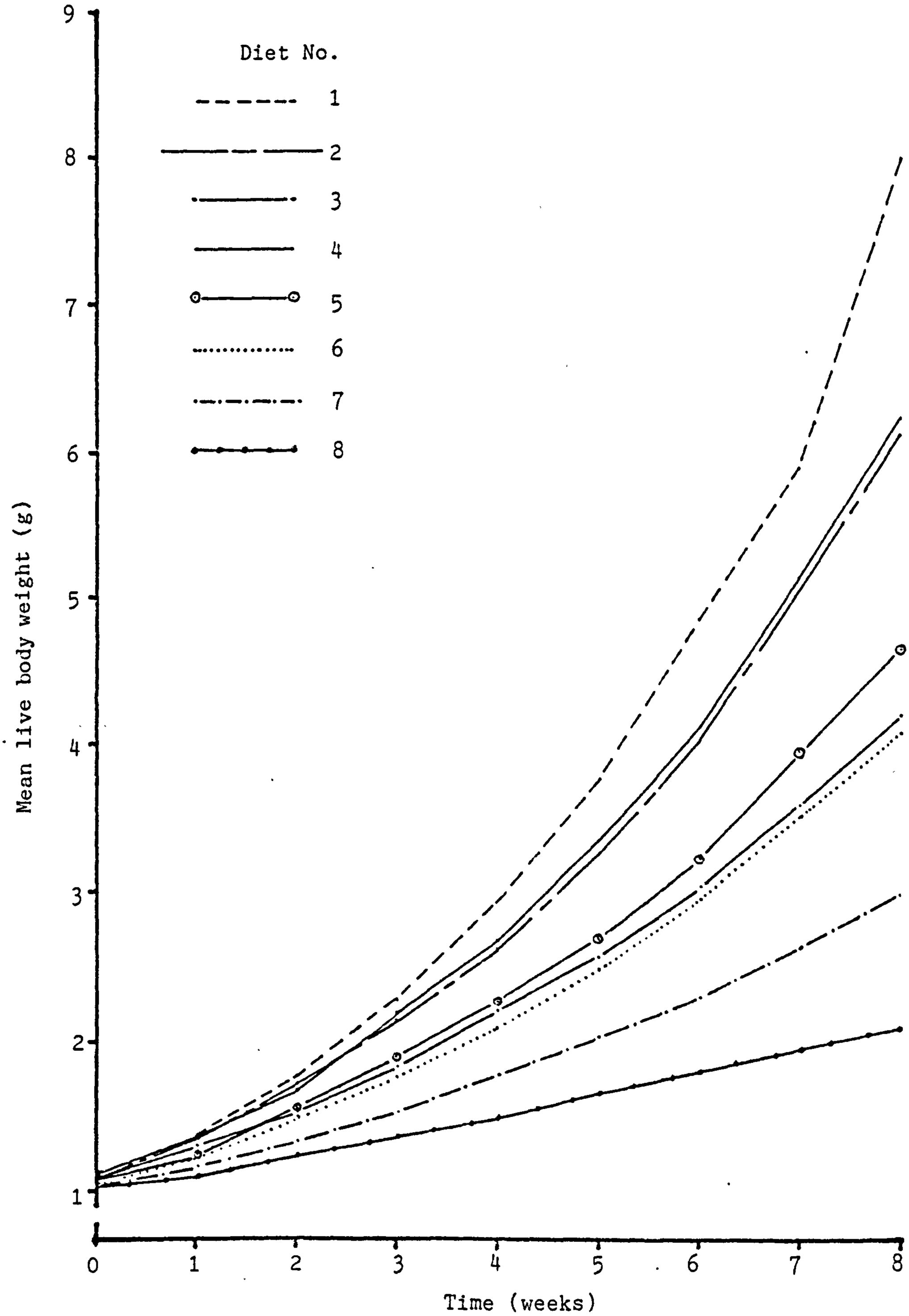


Figure 3.1 Growth responses of fish fed exerpimental diets for 8 weeks

experimental period, while Diet 8 (75% inclusion of sesame) resulted in the poorest growth (Figure 3.1). Out of the plant protein sources tested (except Control diet), Diets 2 (25% mustard) and 4 (25% linseed) resulted in significantly ($p < 0.05$) better growth performances than for all other experimental diets. SGRs ranged from 1.29 to 3.58 (Table 3.9). The SGR's of Diets 2 (25% mustard) and 4 (25% linseed) were not significantly different ($p > 0.05$) but were significantly higher ($p < 0.05$) than SGRs recorded for the rest of the diets. Similarly, there were no significant differences ($p > 0.05$) between the SGR's of Diets 3 (50% mustard) and 5 (50% linseed) but these values were significantly higher ($p < 0.05$) than those of Diets 7 and 8.

3.3.6. Food Conversion Ratio (FCR)

Mean food conversion ratios (FCRs) were obtained for each diet and are presented in Table 3.9. The Control diet had the lowest food conversion ratio (FCR 2.09) among all the experimental diets. Diets 4 (25% linseed) and 2 (25% mustard) were the next most efficient with FCRs of 2.39 and 2.46 respectively. In general, the FCR tended to increase with the increase of plant protein in the diet. FCRs ranged between 2.09 and 6.32. Diet 8 (75% sesame) had significantly the highest ($p < 0.05$), and hence the poorest FCR.

3.3.7. Protein Utilization

Protein utilization efficiency, measured in terms of protein efficiency ratio (PER) and apparent net protein utilization (ANPU), is presented in

Table 3.9. The protein efficiency ratios followed the same trend as the FCRs with the fish meal based Control diet producing significantly ($p < 0.05$) the highest PER followed by Diet 4. Like FCR, there were no significant ($p > 0.05$) difference between the PERs of Diets 2 (25% mustard) and 4 (25% linseed) but these values were significantly ($p < 0.05$) higher than for the other diets (3, 5, 6, 7, 8). PERs ranged from 0.39 to 1.17.

Mean apparent net protein utilization (ANPU %) was calculated for all the dietary treatments and ranged from 5.41 to 17.82 (Table 3.9). The highest value (17.82) was produced by the Control followed by Diet 4. Since the carcass protein contents (see Table 3.11) of various fish groups were similar, the ANPU % values in general tended to reflect the PER value.

3.3.8. Apparent Nutrient Digestibility

Apparent digestibilities of the nutrients in the experimental diets were determined as described in Section 2.8.1 and are presented in Table 3.10. Apparent dry matter digestibility values for different dietary treatments ranged from 50% to 76.21% with the Control diet having the highest value followed by Diet 6. Apparent protein digestibilities for all the diets were fairly high ranging from 77.72% to 89.80%. Again, the fish meal based control diet had the highest (89.80%) protein digestibility. In general, the apparent protein digestibility decreased with the increase of level of plant protein in the diet. The apparent lipid digestibility ranged from 82.0% to 89.31%. The apparent energy digestibility of the experimental diets ranged from 66.40% to 81.12%. The Control diet had

TABLE 3.10

Apparent nutrient digestibility (%) of the experimental diets*

	DIET NO.							
	1 (Control)	2	3	4	5	6	7	8
Apparent dry matter digestibility	76.21	61.54	52.34	59.68	50.00	61.90	53.33	50.96
Apparent protein digestibility	89.80	83.99	81.12	84.85	78.02	81.21	78.40	77.72
Apparent lipid digestibility	89.31	87.35	86.51	89.81	84.52	85.44	83.18	82.01
Apparent ash digestibility	23.49	13.37	16.43	19.84	19.52	14.76	13.66	12.89
Apparent energy digestibility	81.12	70.02	66.50	70.08	64.44	71.64	67.40	66.47

* No statistical analysis was possible as determinations were performed on pooled samples

TABLE 3.11

Proximate carcass composition analysis (% fresh weight basis) of fish samples at the start and the end of the experiment

Components	Initial (all fish)	Final Diet No. 1 (Control)	2	3	4	5	6	7	8	± S.E.[2]
Moisture	80.40	76.68^{d1}	76.85^{d}	78.11^{b}	76.52^{d}	77.56^{c}	77.62^{c}	78.45^{b}	79.71^{a}	0.12
Crude protein	13.14	14.71^{a}	14.51^{ab}	13.80^{c}	14.64^{a}	13.73^{c}	14.19^{b}	13.66^{c}	13.51^{c}	0.12
Crude lipid	3.32	6.17^{a}	6.08^{a}	5.68^{c}	5.81^{b}	5.10^{c}	4.82^{d}	4.54^{e}	3.91^{f}	0.04
Ash	2.28	2.31^{de}	2.27^{e}	2.34^{d}	2.41^{c}	2.47^{ab}	2.48^{ab}	2.45^{bc}	2.51^{a}	0.02
TOTAL	99.14	99.87	99.71	99.93	99.38	98.86	99.11	99.10	99.64	-

[1] Figures in the same row with the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

the highest (81.12%) and Diet 5 (50% linseed) had the lowest energy digestibility. The apparent ash digestibility ranged between 12.89% and 23.49%.

3.3.9. Carcass Composition

Proximate carcass composition of fish at the start and at the end of the experiment is presented in Table 3.11. Fish fed Diet 8 had significantly ($p < 0.05$) the highest moisture content (79.71%) whilst other dietary groups ranged from 76.52% to 78.45%. In general, fish from the diets with higher levels of plant protein inclusion had higher carcass moisture contents and lower lipid contents. The carcass lipid content in different dietary groups ranged from 3.9% to 6.17%. The carcass protein content in different dietary groups ranged from 13.51% to 14.71%. Fish from Diets 1, 2, 4 and 6 had significantly ($p < 0.05$) higher carcass protein contents than other dietary groups but there were no significant ($p > 0.05$) differences between the carcass protein contents of fish fed Diets 1, 2 and 4. The carcass ash content ranged from 2.27% to 2.51%.

3.4 DISCUSSION

The results of the present investigation demonstrated that even 25% replacement of fish meal by oilseed protein resulted in reduced growth rate, poorer food conversion and poorer protein utilization. Several possible explanations for these observations may be offered including:

(1) The effects of different level of anti-nutritional factors in the diets. For example, glucosinolates (allyl isothiocyanate) and tannins in mustard oilcake, phytic acid in sesame and linseed meal and hydrocyanic acid (HCN) in linseed.

(2) Imbalances of limiting essential amino acids such as lysine, methionine, threonine and phenylalanine in the diets containing varying plant protein inclusion levels and sources.

(3) Variation in protein and energy digestibility and availability of minerals and amino acids in the different diets.

(4) Variation in acceptability of the diets.

Despite relatively good acceptability (Table 3.8) and no EAA deficiencies (Table 3.6), Diet 2 containing 25% protein from mustard oilcake produced poor growth responses and food utilization compared to the fish meal based Control diet. This observation is similar to the findings of Capper et al. (1982). These authors found that untreated mustard meal (black mustard from Nepal) included at 20% in the diet of fingerling common carp for 50 days resulted in reduced live-weight gain and adverse changes in the rate of food conversion. Hasan (1986) also found similar poor growth responses of carp fry fed diets containing 25% protein from mustard oilcake. Attempts by Yurkowski et al. (1978) to incorporate more than 30% of the dietary protein as rapeseed meal protein concentrate for rainbow trout led to similar poor growth and food utilization. Hilton and Slinger (1986) reported that canola meal (a low glucosinolate variety of rapeseed) cannot effectively replace soybean meal or fish meal in practical trout diets at levels of 13% or greater without sacrificing growth.

The overall growth performance of fish fed Diet 3 (50% mustard) was significantly ($p < 0.05$) poorer than Diet 2 (25% mustard). However, Diet 3 was slightly deficient in lysine, methionine, phenylalanine and threonine (Table 3.6). Moreover, mustard oilcake used in Diets 2 and 3 contains potentially toxic components, the glucosinolates, which upon hydrolysis by an endogenous enzyme (myrosinase), usually present in the seed or produced by certain microorganisms in the gut, release highly toxic allyl isothiocyanates (Bell and Belzile, 1965; Maheswari et al., 1981). The mustard oilcake used here contained 5.4 mg/g of allyl isothiocyanate which is slightly higher than that (4.0 mg/g) reported by Mustakas et al. (1962) and lower than that (9.4 mg/g) reported by Shah et al. (1977) in mustard oilcake.

In the present experiment Diets 2 and 3 contained 1.6 and 3.3 mg/g of allyl isothiocyanate respectively. These values are considerably higher than the maximum 300µg total glucosinolate/g diet suggested by Higgs et al. (1982) for chinook salmon, and 230-240 µg total glucosinolate/g diet used without effect in trout by Hardy and Sullivan (1983). Although the types and sources of glucosinolates as well as experimental animals reported by Higgs et al. (1982) and Hardy and Sullivan (1983) are different from the present study, the poor growth response of fish fed diets containing mustard oilcake could be attributed to the toxic allyl isothiocyanate content of the diet. The toxic effects of isothiocyanates in rapeseed meal have also been indicated in birds and mammals by thyroid enlargement, depressed growth, reduced utilization of food and histological changes in liver and kidney (Bell and Belzile, 1965; Lo and Hill, 1971).

Moreover, Diets 2 and 3 supplemented with mustard oilcake also contained 0.57% and 1.14% tannins respectively. Vohra et al. (1966) reported that as low as 0.5% dietary tannins caused a depression in the growth of chickens. Tannins have also been reported to lower the metabolizable energy value of rapeseed meal in chickens (Yapar and Clandinin, 1972). Although there is no published information available on the toxicity of tannins to fish, the tannin content in Diets 2 and 3 might have partially contributed to the poorer growth responses.

With respect to acceptability, growth, food conversion and protein utilization, the performance of carp fed Diet 4 (25% linseed) was similar to Diet 2 (25% mustard) but significantly ($p < 0.05$) higher than Diet 5. However, both Diets 4 (25% linseed) and 5 (50% linseed) were deficient in some EAAs (Table 3.6). Diet 4 had a lysine content of 1.98% and Diet 5 (50% linseed) 1.96% whereas the requirement of carp is 2.12% (after Ogino, 1980). Similarly, Diets 4 and 5 were also deficient in methionine and threonine (Table 3.6). The deficiency of the above mentioned EAAs in Diets 4 and 5 could partially be responsible for the poor growth performances observed. The efficiency of linseed meal as a dietary protein supplement for domestic farm animals has been investigated fairly extensively (Montgomery, 1980; Gohl, 1981; MacDonald, et al., 1981). Many workers have reported that linseed meal causes significant growth depression in chicks even after correcting the amino acid deficiencies in the diet by adequate amino acid supplementation (McGregor and McGinnis, 1948; Mandokhot and Singh, 1979).

The only attempt to use linseed meal as a protein source for fish appears to have been by Hasan (1986). The author used linseed meal as 25% dietary protein replacement for fish meal in carp fry diets and found no significant differences in growth performance compared to the Control. The better growth performance of fish reported by Hasan, (1986) compared to the present study on diets supplemented with 25% linseed protein could probably be due to the differences in source, proximate composition, and anti-nutritional factors of linseed and as well as the size differences of the experimental fish used. For example, Hasan (1986) used small fry of mean weight 0.106g compared to that of 1.06g used in the present study.

Linseed meal contains the cyanogenetic glucoside, linamarin, which in the presence of a heat labile autoenzyme linamarase liberates hydrogen cyanide (HCN) on hydrolysis (Montgomery, 1964). However, processing involving high temperature treatment destroys most of the linamarin and the enzyme (Linamarase) and the resultant meals are quite safe (Gohl, 1981; Aquaculture Development Coordination Programme ADCP, 1983).

Diets 4 and 5 in this present trial were supplemented with linseed meal in the form of expeller cake (see Section 3.2.2) and contained 0.13 and 0.26 mg/g of HCN (Table 3.5) respectively. These two diets (4 and 5) also contained 0.78% and 1.56% of phytic acid respectively. The growth depression of carp fed these diets presumably might be due to the presence of the above mentioned anti-nutritional factors, most likely the phytic acid content in the diets. Spinelli et al. (1983) also found 10% reduction in growth and food conversion of rainbow trout fed diets

containing only 0.5% phytic acid for 105 days. Various authors have suggested that growth depression in chicks might be due to the presence of cyanogenetic glucosides, phytic acid and anti-pyri doxine factors in linseed (Kratzer, 1947; Mani et al., 1949; Mandokhot and Singh, 1979; Madhusudhan and Singh, 1983).

Diet 6 containing 25% of the total protein from sesame meal did not produce comparable growth to that of Diet 2 (25% mustard) and Diet 5 (25% linseed). All the diets (6, 7, 8) containing different inclusion levels of sesame meal produced significantly lower growth than for the rest of the protein sources at the same level of inclusion.

The above observations corroborate the findings of Hasan (1986) who found similarly poor growth responses in carp fry fed diets containing various levels of sesame meal. Richards (1983) also observed reduced growth rate with Oreochromis niloticus fed diets containing 25% sesame meal compared to a fish meal based control diet. In contrast, ADCP (1983) reported that experiments on Indian carp (Labeo rohita) have shown that up to 50% of sesame oilcake may be fed in a complete diet with good results. Several workers have observed poor growth performance of chicks fed sesame meal (Almquist and Grau, 1944; Lease, 1966). However, the effects of use of high levels of sesame meal have not been studied in fish although in conventional livestock, it has been shown to have a laxative effect (ADCP, 1983).

Sesame meal has a high phytic content (Gohl, 1981; ADCP, 1983). Phytic acid forms strong complexes with proteins and di- and tri-valent metal

ions to form insoluble salts at the pHs occurring in the intestine of fish and other monograstric animals (Liener, 1977; Erdman, 1979) and therefore rendering them unavailable for absorption. The ability of phytic acid to bind metal ions is lost when the phosphate groups are hydrolysed through the action of the enzyme phytase (Liener, 1977). Animals such as fish, with simple stomachs lack the enzyme phytase in the gastrointestinal tract (Lall, 1979). Therefore fish cannot utilize phytate bound phosphorous or other metal ions.

In the present experiment, Diets 6, 7 and 8 contained 0.66%, 1.33% and 1.99% of phytic acid. These high levels of phytic acid may have been in part responsible for the observed poor growth performances of carp fry. Richardson et al. (1985) reported similar findings in juvenile chinook salmon. These authors reported that high phytic acid levels (2.58%) depressed growth, food and protein conversion (PER) and thyroid function.

Diet 6 (25% sesame) was slightly deficient in lysine but both Diets 7 and 8 were deficient in lysine, methionine and phenylalanine (Table 3.6). The available lysine content of the diets containing sesame meal was comparatively lower than the diets containing either mustard or linseed meals (see Table 3.5). This deficiency of EAAs in the diets containing high levels of sesame meal protein may have been another contributing factor to the lower growth responses of fish observed.

Apart from the high fish meal based Control diet all the test protein sources in combination with fish meal showed comparatively low dry matter digestibility (Table 3.10). The dry matter digestibility values tended

to decrease with the increase of plant protein levels in the diets.

In the present study, the fish meal diet showed highest apparent protein digestibility (89.80%) among all the dietary groups which is in agreement with the values reported for carp and other species by various authors (Kim, 1974; Smith et al., 1980; Brown et al., 1985). Kim (1974) reported an apparent protein digestibility (APD) value of 88.8% for fish meal in carp. According to NRC (1977) carp can digest 95% of fish meal protein. However, the value can decrease to 80%-85% depending on the origin and processing of fish meal (Ogino and Chen, 1973). The APD values of other diets varied between 77.72% to 84.85% with Diet 2 (25% mustard) having the highest and Diet 8 lowest (Table 3.10). These APDs are comparable to those found by Hasan (1986) in common carp fry fed diets containing different proportions of mustard, linseed and sesame meal protein. However, Smith et al. (1980) reported a slightly lower APD of 76.7% for linseed used as a sole source of protein for rainbow trout.

Diet 5 containing 50% linseed meal gave a low APD similar to Diet 8 (75% sesame). This low digestibility in Diet 5 might be due to the presence of mucilage in linseed meal. Mani et al. (1949) reported that mucilage of the linseed hull might affect digestibility because of the viscous nature of the wetted material. Addition of linseed mucilage to the normal ration produced a retardation in growth of chicks similar to the effect of untreated meal.

The comparatively low protein digestibility of the diets (6, 7 and 8) containing differing levels of sesame meal may have been due to the

presence of phytic acid. Spinelli et al. (1983) reported that phytic acid (at 0.5%) reduced protein digestibility in rainbow trout.

The lipid digestibility of all the diets was as high as that of crude protein but not as high as reported for carp by NRC (1977). This might be because the crude lipid fraction in plant protein represents a very heterogenous group of compounds. Pure lipid either in the form of vegetable oil (e.g. soybean) or fish oil are over 90% digested by carp (Takeuchi et al., 1979). The composition of the fatty acids and the melting point of the lipid have a strong influence on digestibility.

The fish meal based Control diet showed significantly ($p < 0.05$) the highest (81.12%) apparent energy digestibility (AED) among the experimental diets. This AED is lower than the reported value of fish meal for trout (91.5%) by Smith et al. (1980) and higher than that for trout (74%) by Windell et al. (1978b) but similar to that of grass carp (83%) by Law (1986). The AEDs of the other diets varied between 66.4% to 71.64% (Table 3.10). The AEDs found here with the diets containing different plant protein sources are lower than that found with dehulled soybean (75%) by Cho et al. (1982) for trout but similar to that of linseed meal (71%) and soybean (70.8%) for trout by Smith et al. (1980).

The fish that showed greatest weight increases tended to have lower carcass moisture but higher protein and lipid content when compared to those fed diets containing higher levels of plant protein (Table 3.11). However, carcass moisture and lipid content showed large fluctuations. Such fluctuations appear to be inversely related as are also reported by

other authors (Andrews and Stickney, 1972; Takeuchi et al., 1978; Atack et al., 1979; Zeitlar et al., 1984). In this study the diets containing higher levels of plant protein inclusion produced significantly ($p < 0.05$) lower carcass lipid (Table 3.11). This observation is in agreement with Dabrowski and Kozlowska (1981), Yurkowski et al. (1978) and Higgs et al. (1979) who observed similar reduction in carcass lipid in carp, rainbow trout and coho salmon respectively, fed diets containing rapeseed meal. Appler and Jauncey (1983) also observed marked decreases in carcass lipid content with O. niloticus fed diets containing filamentous green algae as a partial or sole source of dietary protein.

The histological examination of various tissues at the end of the experiment showed changes in the liver and thyroid tissues of the fish fed diets containing 50% mustard oilcake (see Section 3.3.4). These changes in thyroid tissue might be due to the presence of glucosinolates (allyl isothiocyanate) in mustard oilcake. The trend to increased epithelial cell heights in carp fed diets containing higher level of mustard oilcake could be an adaptation of goitrogen activity. Higgs et al. (1979) reported that in vertebrates dietary rapeseed meal can induce thyroid hypertrophy, depressed growth, feed intake and utilization and histological changes in the liver, kidney and thyroid.

The histological changes in liver (intracellular fat deposition) of fish fed Diet 3 (50% mustard) may be due to the presence of glucosinolate (allyl isothiocyanate). Similar lipid deposition in the liver of rat and coho salmon was observed by Oliver et al. (1971) with diets containing rapeseed meal with low and high glucosinolates. However, in the present

study, although fish fed Diet 3 (50% mustard) showed a high level of intracellular fat deposition, the carcass lipid (5.68%) was significantly ($p < 0.05$) lower than the control (6.17%).

The results of this study indicate that the above mentioned plant protein sources cannot be used as a replacement for fish meal at a level of 25% or more of the dietary protein without sacrificing growth and feed efficiency.

CHAPTER 4 : EXPERIMENT 2

Effect of essential amino acid supplementation on the growth and feed utilization in common carp (*C. carpio* L)

4.1 INTRODUCTION

The high price and increasing scarcity of fish meal has made necessary the search for substitutes in fish feed. A possible solution to this problem is being sought in the utilization of plant proteins, mainly oilseed meals. However, apart from fish meal there are few animal or plant protein products available for fish feed formulation with an essential amino acid (EAA) profile approximating the dietary EAA requirements of farmed fish.

Numerous attempts have been made to partially or completely replace varying proportions of fish feed with oilseed meals. These have met with variable success (Atack et al., 1979; Jackson et al., 1982; Murai et al., 1982b; Viola et al., 1982). Compared to fish meal, which has a well balanced amino acid profile, the majority of the plant protein sources tested were either deficient in one or more essential amino acids. In addition the presence of disproportionate levels of specific amino acids may lead to amino acid antagonisms. Examples of such antagonisms are leucine/isoleucine and arginine/lysine (Tacon and Jackson, 1985). Despite these amino acid imbalances, some researchers (Higgs et al., 1979) have been successful in utilizing such feed proteins by supplementing them with other proteins so as to obtain the required EAA profile for fish.

Reports on the value of supplementing deficient diets, for a variety of fish species, with crystalline amino acids are contradictory. Andrews and Page (1974) observed reduced growth in young channel catfish when soybean meal was supplemented with methionine, cystine or lysine. On the contrary, Robinson et al. (1980) reported that peanut (groundnut) meal

diets(formulated to be first limiting in lysine) supplemented with feed grade lysine demonstrated that catfish are able to utilize free amino acids effectively.

Aoe et al. (1970) found that young carp (C. carpio) showed little growth on diets in which the protein component (casein and gelatin) was entirely replaced by a mixture of amino acids. Studies with fingerling carp and channel catfish (Murai et al., 1981; 1982a) showed that the efficiency of utilization of crystalline amino acids supplemented to gelatine and utilization of amino acid mixtures could be drastically enhanced by adjusting pH and coating them with casein. In a subsequent study Murai et al. (1982b) reported that the supplementation of coated and uncoated methionine to a soybean based diet significantly improved growth and feed efficiency in carp and channel catfish. In carp feed, addition of methionine coated with aldehyde treated casein significantly enhanced the growth over that of uncoated methionine.

In a recent study Murai et al. (1986) concluded that the nutritive value of soyflour for carp was improved by addition of essential amino acids, especially methionine. Yamada et al. (1981) showed that by increasing the feeding frequency of the amino acid test diets growth and feed efficiency in carp were dramatically improved, nearly equalling those of carp fed a casein control diet. In studies on the apparent digestibility of amino acids in carp Plakas and Katayama (1981) found that this species absorbed amino acids more rapidly from an amino acid test diet than from a purified protein diet.

In the previous experiment (Table 3.6) most of the diets supplemented with plant proteins were deficient in one or more essential amino acids and resulted in reduced growth of carp compared to the control. The objective of the present experiment was to determine whether adding crystalline amino acids improved the nutritive value of diets fed to carp fry. Furthermore an attempt was made to investigate the effect of supplementation of the first limiting amino acid alone and in combination with other limiting amino acids on the utilization of plant protein feeds by carp.

4.2 MATERIALS AND METHODS

4.2.1. Experimental Systems and Animals

The experimental systems used in the present study are described in detail in Section 2.1.1 and 2.1.2. The sources of experimental animals and their quarantine procedures are described in Section 2.2.1.

4.2.2. Analytical Techniques

The analytical techniques used for proximate composition, calcium, phosphorous, available lysine, amino acids, chromic oxide and anti-nutritional factors in ingredients, diets, faeces and carcass samples were as used in Experiment 1. All these techniques have been described in detail in Chapter 2. Dietary tryptophan levels were calculated from data for the ingredients used in this experiment using data from NRC (1983) except for mustard oilcake which was obtained from Capper et al. (1982).

4.2.3. Diet Formulation and Preparation

Ten semi-purified isonitrogenous diets were formulated using the same protein sources as in Experiment 1 (Section 3.3.2). The origins of the oilseeds were as for Experiment 1. In view of the very poor growth of carp fed the 75% sesame meal diet in Experiment 1 (Table 3.9), it was decided to limit plant protein source inclusion to a maximum of 50% in the present experiment.

In the present experiment mustard oilcake was only used at the 50% inclusion level because at 25% the formulation was not deficient in any of the essential amino acids (see Table 3.6). Limiting EAAs were calculated by comparing the EAA composition of the diets in Experiment 1 (Table 3.6) with the requirement for carp after Ogino et al. (1980).

Feed formulation and preparation were as for Experiment 1, except that the deficient diets were supplemented with amino acids by replacement of α-cellulose. The crystalline amino acids (L-form) were obtained from Sigma Chemical Co Ltd, Poole, Dorset, England. Diets were supplemented either with the first deficient amino acid or with a combination of all the deficient amino acids. The series of diets supplemented with first limiting amino acid were designed "A" and the diets supplemented with all the deficient amino acids "B".

Proximate composition, Ca, P, available lysine, anti-nutritional factors and amino acids in the test protein sources were as for Experiment 1

TABLE 4.1.

Level of inclusion of different test proteins (as % of total protein) and supplemental amino acids in the diets used in Experiment 2

	DIET NO.									
	1 (Control)	2A	2B	3A	3B	4A	4B	5A	6A	6B
Herring meal	100	50	50	75	75	50	50	75	50	50
Mustard oilcake	-	50	50	-	-	-	-	-	-	-
Linseed meal	-	-	-	25	25	50	50	-	-	-
Sesame meal	-	-	-	-	-	-	-	25	50	50
First limiting amino acids		Methionine	-	Lysine	-	Methionine	-	Lysine	Lysine	-
Limiting amino acids			Methionine Lysine Phenyl-alanine Threonine		Lysine Threonine Methionine Phenyl-alanine		Lysine Methionine Threonine			Lysine Methionine Phenyl-alanine

A = Diet supplemented with first limiting amino acid

B = Diet supplemented with all the limiting amino acids

TABLE 4.2

Compositon (%) of the experimental diets used in Experiment 2

	DIET NO.									
	1 (Control)	2A	2B	3A	3B	4A	4B	5A	6A	6B
Mustard oilcake	-	61.00	61.00	-	-	-	-	-	-	-
Linseed meal	-	-	-	29.75	29.75	59.25	59.25	-	-	-
Sesame meal	-	-	-	-	-	-	-	23.25	46.50	46.50
Fish meal	52.50	26.25	26.25	39.25	39.25	26.25	26.25	39.25	26.25	26.25
Cod liver oil	0.23	1.14	1.14	1.43	1.43	2.61	2.61	1.43	2.62	2.62
Soybean oil	5.00	-	-	3.12	3.12	1.26	1.26	3.22	1.43	1.43
Mineral premix[1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin premix[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Binder (CMC)[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Dextrin	25.77	2.97	2.79	12.95	12.95	0.63	0.63	18.35	10.70	10.70
α-cellulose	8.00	-	-	4.86	4.67	1.34	1.18	5.92	3.71	3.58
L-Lysine	-	-	0.08	0.14	0.14	0.16	0.16	0.08	0.29	0.29
L-Methionine	-	0.14	0.14	-	0.04	-	0.11	-	-	0.07
L-Phenylalanine	-	-	0.06	-	0.03	-	-	-	-	-
L-Threonine	-	-	0.04	-	0.12	-	0.05	-	-	0.06
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1] For composition of mineral and vitamin premixes see Table 2.3 and 2.4 respectively

[2] Sodium carboxymethyl cellulose (high viscosity)

TABLE 4.3

Analysed proximate composition of the experimental diets used in Experiment 2
(% dry matter basis unless otherwise stated)

	DIET No.									
	1 (Control)	2A	2B	3A	3B	4A	4B	5A	6A	6B
Dry matter	93.42	91.89	91.36	93.30	93.33	93.10	92.83	93.46	92.89	92.47
Crude protein	41.08	40.38	40.80	39.96	40.30	39.98	41.12	39.80	40.48	40.61
Crude lipid	10.12	9.88	10.13	9.66	9.96	9.72	10.08	9.58	9.47	9.52
Crude fibre	7.65	7.65	6.46	7.75	7.70	6.89	6.36	7.69	6.66	6.28
Ash	9.88	12.57	12.62	10.48	10.53	11.12	10.98	11.36	12.69	12.64
NFE[1]	31.27	29.52	29.99	32.15	31.51	32.29	31.46	31.57	30.70	30.95
Ca	1.72	1.65	1.67	1.61	1.62	1.65	1.62	1.70	1.76	1.75
P	1.61	1.66	1.64	1.62	1.63	1.60	1.57	1.64	1.70	1.69
Total energy (Kcal/g)	4.51	4.41	4.43	4.46	4.49	4.48	4.52	4.44	4.43	4.46
PE Ratio[2]	91.08	91.56	92.09	89.60	89.75	89.24	90.97	89.64	91.37	91.05
Allyl isothiocyanate[3]	-	0.33	0.33	-	-	-	-	-	-	-
Phytic acid[3]	-	-	-	0.78	0.78	1.56	1.56	0.66	1.33	1.33
HCN[3]	-	-	-	0.013	0.013	0.026	0.026	-	-	-
Tannins[3]	-	1.14	1.14	-	-	-	-	-	-	-
Chromic Oxide	0.46	0.50	0.52	0.50	0.51	0.51	0.50	0.49	0.49	0.50
Available lysine	2.30	1.77	1.83	1.86	1.86	1.81	1.80	1.85	1.79	1.78
(% of total lysine)	(91.26)	(86.00)	(85.51)	(87.32)	(86.91)	(84.58)	(84.11)	(85.25)	(83.25)	(83.17)

[1] Nitrogen free extractives, calculated as
100-(Moisture+Crude protein+Crude lipid+Ash+Crude fibre)

[2] Protein to energy ratio in mg protein/Kcal of total energy

[3] Level of anit-nutritional factors were estimated from their levels in dietary protein sources as described earlier (Table 3.1)

TABLE 4.4

Analysed essential amino acid (EAA) composition of the experimental diets used in Experiment 2 (% dry matter basis)

Amino acids	DIET NO.										Requirement for carp[a]
	1 (Control)	2A	2B	3A	3B	4A	4B	5A	6A	6B	
Arginine	2.05	2.11	2.13	2.14	2.16	2.82	2.84	2.74	3.05	3.07	1.52
Histidine	0.73	0.82	0.81	0.72	0.71	0.72	0.73	0.83	0.68	0.67	0.56
Isoleucine	1.42	1.28	1.27	1.24	1.24	1.28	1.27	1.61	1.32	1.31	0.92
Leucine	2.52	2.35	2.34	2.15	2.16	2.30	2.29	2.82	2.38	2.38	1.64
Lysine	2.52	2.06 (97.2)[b]	2.14	2.13	2.14	1.96 (92.5)	2.14	2.17	2.15	2.14	2.12
Methionine	0.93	0.66	0.66	0.62 (96.9)	0.66	0.65	0.65	1.10	0.60 (93.7)	0.66	0.64
Cystine	0.24	0.23	0.24	0.22	0.23	0.40	0.40	0.31	0.29	0.30	-
Phenylalanine	1.21	1.12 (96.5)	1.17	1.12 (96.5)	1.18	1.33	1.34	1.53	1.64	1.66	1.16
Tyrosine	0.83	0.81	0.83	0.65	0.66	0.85	0.84	1.12	0.95	0.94	-
Threonine	1.44	1.28 (96.9)	1.34	1.23 (93.2)	1.34	1.26 (95.5)	1.33	1.78	1.28 (96.9)	1.35	1.32
Tryptophan[c]	0.44	0.53	0.53	0.49	0.49	0.48	0.48	0.50	0.57	0.57	0.24
Valine	1.60	1.68	1.66	1.54	1.55	1.65	1.64	2.02	1.75	1.72	1.16

[a] Data for EAA requirement of carp from Ogino (1980)

[b] Figures within parenthesis indicate chemical scores of deficient EAA

[c] Estimated from levels in the dietary protein sources as described earlier (Table 3.2)

TABLE 4.5

A summary of the methodology used to study the effect of supplementation of limiting dietary essential amino acids (EAA) on growth and feed utilization in carp

Fish	C. carpio average initial weight 1.79 ± 0.02g
Duration of Experiment	Eight weeks
Treatments	Supplementation of EAA in diets either as first deficient EAA alone or in combination of all deficient EAA to determine whether addition of EAA improves the nutritive value of these diets for carp
No. of Treatments	10
Replication	3/treatment
Water Temperature	27° ± 1°C
Stocking Density	15/tank
Water Flow Rate	1 litre/min/tank
Carcass Sampling	Initial sample - 10 fish at the start of the experiment Final sample - 12 fish per treatment (4 from each replicate)
Faeces Collection	Collected twice daily for two weeks dried at 60°C in an oven and used for digestibility study
Physico chemical characteristics monitored	Temperature, pH, dissolved oxygen and total ammonia

(Tables 3.2 and 3.3). The levels of inclusion of test protein sources and supplemental amino acids are shown in Table 4.1. The formulations of the experimental diets are presented in Table 4.2.

All diets were analysed for proximate composition, Ca, P and available lysine, results are shown in Table 4.3. The amino acid contents of the diets were analysed and are presented in Table 4.4. Levels of anti-nutritional factors in the diets were calculated using respective analysed values for the individual plant protein sources.

4.2.4. General Experimental Procedure

There were three replicates for each treatment with 15 fish in each replicate. The acclimation and weighing procedures were as for Experiment 1 (Section 2.2.3). Fish were bulk weighed weekly after 12 hour starvation during the experimental period. The experiment was conducted for a period of eight weeks. A summary of the methodology used in the present experiment is given in Table 4.5.

4.2.5. Feeding Rates

Fish were fed three times daily between 09.00 and 17.00 hours at four hourly intervals. Fish were fed 10% of their body weight per day at the beginning of the experiment, which was reduced to 6% per day after the fourth week. Details of feeding rate and food administration were as for Experiment 1 (Section 3.2.5). The quantity of food delivered per day was adjusted after each weekly weighing and fed for the subsequent week.

4.2.6. Faeces Collection

Fish were transferred to the faecal collection system (Section 2.1.2) at the beginning of the seventh week and faeces were collected twice daily, morning and evening, for two weeks. Details of the faecal collection procedure are as for Experiment 1 (Section 3.2.5). Collected faeces were dried in an oven at 60°C. Faecal samples from each replicate tank were pooled to represent respective treatments and kept in air-tight containers for subsequent chemical analysis.

4.2.7. Histological Techniques

The histological techniques used were as for Experiment 1 (Section 3.2.7). Twelve fish from each treatment were used for histological study to assess changes in gills, thyroid, liver, muscle, kidney and intestine.

4.2.8. Analysis of Experimental Data

Analyses of the results were as described earlier (Section 2.8.1). Statistical analyses were performed as described in Section 2.9.1.

4.3 RESULTS

Both the Control and diets containing 25% plant protein (Diets 3A, 3B) were well accepted and fish consumed food aggressively throughout the experiment. However, diets containing 50% plant protein (Diets 2A, 2B,

4A, 4B, 6A and 6B) were less well accepted. No mortality occurred during the experiment.

4.3.1. Analysed Composition of the Diets

Proximate analyses of diets are shown in Table 4.3. Moisture contents varied between 6.54% and 8.64%. Protein levels in all diets were similar and ranged from 39.08% to 41.12%. Lipid, crude fibre and energy contents showed little variation between diets. Levels of anti-nutritional factors in the various diets were similar to those of Experiment 1.

Although overall composition of the diets in this experiment was the same as that of Experiment 1, the available lysine contents were relatively higher. This increase was proportionate to the amount of free lysine supplemented to the diets. Because of supplementation of the experimental diets either with the first limiting EAA or all deficient EAAs, some of the diets (2A, 3A, 4A and 6A) were still deficient in certain EAAs (Table 4.4).

4.3.2. Growth

Growth responses of fish and feed utilization efficiencies are shown in Table 4.6 for the eight week growth study. The growth response of fish is also shown graphically in Figure 4.1. The mean initial weights were insignificantly ($p > 0.05$) different. The fish meal based Control diet produced the best growth responses followed by Diets 3B, 3A, 5A, 4B, 4A, 2B, 2A, 6B and 6A respectively.

TABLE 4.6

Growth and food utilization of common carp fry fed the experimental diets for eight weeks

	Mean Values DIET NO.										±S.E.[2]
	1 (Control)	2A	2B	3A	3B	4A	4B	5A	6A	6B	
Initial weight (g)	1.76^{a}	1.80^{a}	1.80^{a}	1.79^{a}	1.78^{a}	1.81^{a}	1.78^{a}	1.81^{a}	1.79^{a}	1.81^{a}	0.01
Final weight (g)	13.22^{a}	7.40^{f}	7.75^{ef}	12.13^{c}	12.81^{b}	7.49^{f}	7.92^{e}	9.50^{d}	6.28^{g}	6.45^{g}	0.11
Weight gain (g)	11.46^{a}	5.60^{g}	5.95^{ef}	10.34^{c}	11.03^{b}	5.68^{fg}	6.14^{e}	7.69^{d}	4.42^{h}	4.64^{h}	0.09
% weight gain	651^{a}	311^{g}	331^{f}	578^{c}	620^{b}	313^{g}	345^{e}	425^{d}	247^{i}	256^{h}	2.23
SGR (% day)	3.60^{a}	2.52^{g}	2.61^{f}	3.42^{c}	3.52^{b}	2.53^{g}	2.66^{e}	2.96^{d}	2.22^{i}	2.27^{h}	0.01
SGR as % of Control	100	70.00	72.50	95.00	97.77	70.27	73.88	82.22	61.66	63.05	-
FCR	2.09^{g}	2.96^{c}	2.89^{d}	2.22^{f}	2.18^{f}	3.13^{b}	2.94^{cd}	2.63^{e}	3.63^{a}	3.61^{a}	0.01
PER	1.16^{a}	0.84^{c}	0.85^{c}	1.13^{a}	1.14^{a}	0.79^{d}	0.83^{c}	0.95^{b}	0.68^{e}	0.71^{e}	0.01
ANPU (%)	17.08^{ab}	12.25^{e}	12.72^{d}	16.99^{b}	17.18^{a}	11.63^{f}	12.14^{e}	13.92^{c}	9.96^{h}	10.36^{g}	0.04

[1] Figures in the same row having the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

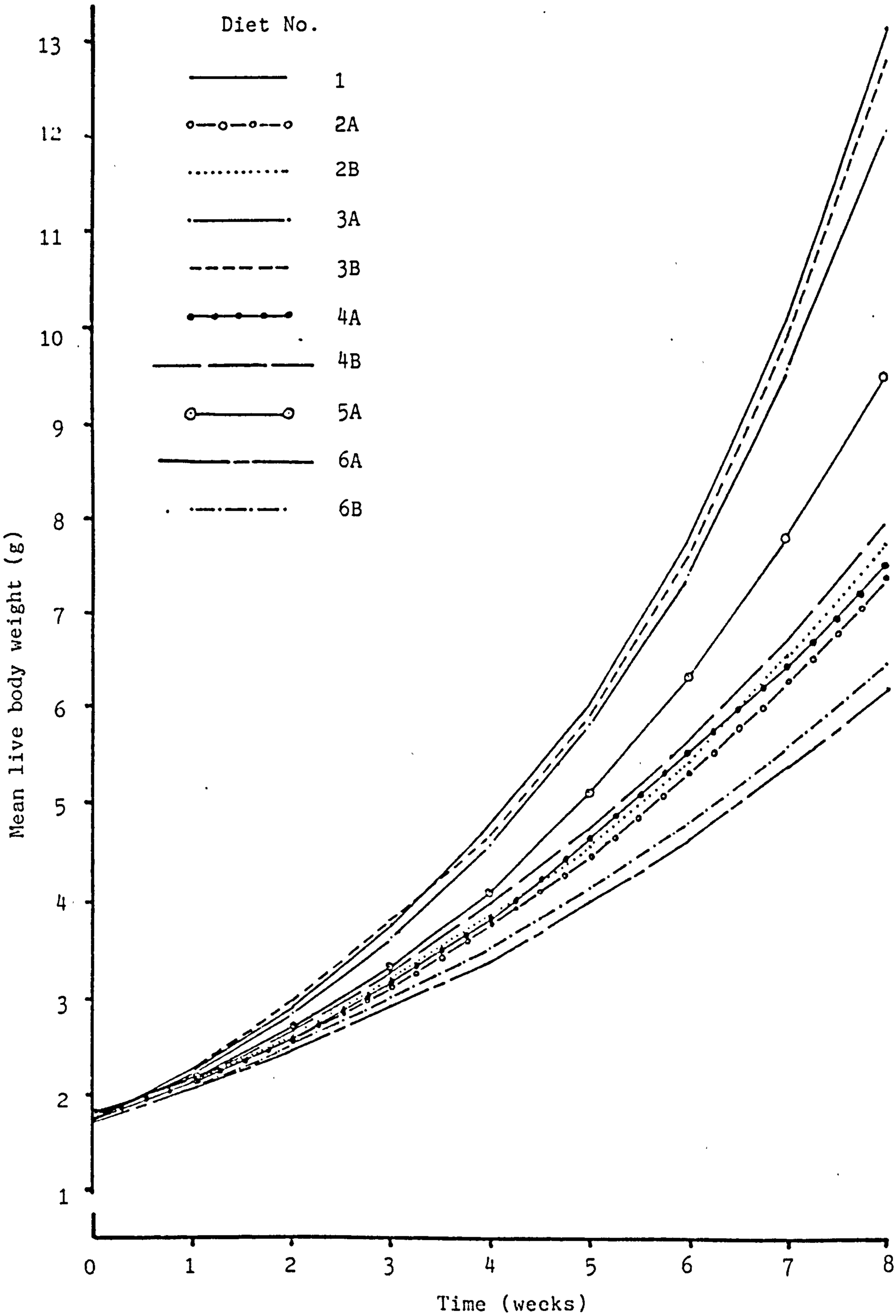


FIGURE 4.1 Growth responses of fish fed experimental diets for 8 weeks

In all cases fish fed diets supplemented with all the deficient EAA (Series B) produced better growth than fish fed diets supplemented only with the first limiting EAA (Series A). In the case of diets containing different levels of linseed protein, the growth responses of fish fed diets supplemented with all the deficient EAA (Diets 3B and 4B) were significantly ($p < 0.05$) higher than for fish fed diets supplemented only with the first limiting EAA (Diets 3A and 4A).

Statistical analysis of the specific growth rates (SGR) presented in Table 4.6 showed that the Control diet gave significantly ($p < 0.05$) the highest growth response (SGR 3.60) and Diet 6A (50% sesame protein) the lowest (SGR 2.22). Out of the plant protein sources tested (except Control), Diet 3B (supplemented with all deficient EAA) produced a significantly ($p < 0.05$) better SGR than the rest of the diets. Growth on Diet 3B was about 97.77% of the SGR of the Control. There were no significant differences ($p > 0.05$) in SGR between Diets 2A and 4A (50% mustard protein and 50% linseed protein respectively) supplemented only with the first limiting EAA.

4.3.3. Food Conversion Ratio (FCR)

Mean food conversion ratios (FCR) obtained for each experimental diet is presented in Table 4.6. The FCR values were relatively high ranging from 2.09 in the Control to 3.63 in Diet 6A (50% sesame protein). There were no significant ($p > 0.05$) differences between the FCRs of Diets 2A and 4B, 3A and 3B and 6A and 6B respectively. In general, FCRs increased with increasing level of plant protein in the diet.

4.3.4. Protein Utilization

The efficiency with which fish utilized dietary protein was determined by calculation of protein efficiency ratio (PER) and apparent net protein utilization (ANPU) (Table 4.6). PERs ranged from 0.68 to 1.16, Diets 6A and 6B gave significantly ($p < 0.05$) the poorest PERs. There were no significant ($p > 0.05$) differences between the PERs of Diets 1 (Control), 3A and 3B but these values were significantly ($p < 0.05$) higher than for the rest of the diets. Similarly, there were no significant differences in PERs between Diets 2A, 2B and 4B although these were significantly ($p < 0.05$) higher than for the Diets 4A, 6A and 6B.

Mean apparent net protein utilization (ANPU %) was calculated for all dietary treatments and is presented in Table 4.6. The ANPU values ranged from 9.96 to 17.18 and Diet 6A gave significantly ($p < 0.05$) the lowest ANPU. There were no significant differences between the ANPUs of Diets 1 and 3B but these values were significantly ($p < 0.05$) higher than for the rest of the diets. Again, there were no significant ($p > 0.05$) differences between ANPU values for Diets 1 and 3A. In general the ANPU values reflected the PER.

4.3.5. Apparent Nutrient Digestibility

Apparent nutrient digestibilities of the experimental diets were determined as described in Section 2.8.1 and are presented in Table 4.7. The apparent dry matter digestibilities for different dietary treatments

TABLE 4.7

Apparent nutrient digestibility (%) of the experimental diets

	DIET NO.									
	1 (Control)	2A	2B	3A	3B	4A	4B	5A	6A	6B
Apparent dry matter digestibility	69.74	55.36	56.30	59.34	62.77	54.46	55.35	56.25	48.95	48.98
Apparent protein digestibility	87.04	82.25	82.68	84.95	83.48	82.49	82.70	79.76	79.57	78.30
Apparent lipid digestibility	90.04	86.21	86.82	90.29	88.56	86.72	87.08	87.62	85.33	85.21
Apparent ash digestibility	22.62	15.26	15.90	20.21	20.84	19.28	18.56	16.67	14.31	14.10
Apparent energy digestibility	82.20	67.80	68.92	73.45	74.60	68.16	69.00	72.48	67.66	68.48

*No statistical analysis was possible as determinations were performed on pooled samples

TABLE 4.8

Proximate carcass composition analysis (% fresh weight basis) of fish samples at the start and end of the experiment

	DIET NO.											±S.E.[2]
	Initial all fish	1	2A	2B	3A	3B	4A	4B	5A	6A	6B	
Moisture	80.17	77.68^{d} [1]	79.80^{a}	79.31^{b}	77.60^{d}	77.58^{d}	79.60^{ab}	79.45^{ab}	78.46^{c}	79.36^{ab}	79.46^{ab}	0.14
Crude protein	13.20	14.58^{bc}	14.31^{d}	14.59^{bc}	14.81^{ab}	14.84^{a}	14.30^{d}	14.37^{cd}	14.35^{d}	14.17^{d}	14.25^{d}	0.07
Crude lipid	3.35	5.09^{a}	2.96^{d}	3.13^{d}	4.72^{b}	4.56^{c}	3.12^{d}	3.12^{d}	4.38^{c}	3.14^{d}	3.11^{d}	0.06
Ash	2.29	2.28^{b}	2.55^{a}	2.51^{a}	2.36^{b}	2.27^{b}	2.51^{a}	2.54^{a}	2.33^{b}	2.62^{a}	2.58^{a}	0.03
TOTAL	99.01	99.63	99.62	99.54	99.49	99.25	99.53	99.48	99.52	99.29	99.40	

[1] Figures in the same row with the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of treatment means calculated from the residual mean square in the analysis of variance

were relatively low and ranged between 48.95% (Diet 6A) and 69.74% (Diet 1). Most of the diets showed good apparent protein digestibility values ranging from 78.30% to 87.04%. In general the apparent protein digestibility decreased with increasing plant protein level in the diet. The apparent lipid digestibility values ranged from 85.21% to 90.40% which are relatively higher than in Experiment 1 (Section 3.3.8). The apparent ash digestibility values ranged from 14.10% to 22.62%. The apparent energy digestibility values were comparatively higher than in Experiment 1 (Section 3.3.8) and ranged from 67.66% to 82.20%.

4.3.6. Carcass Composition

Proximate carcass composition data for fish at the start and at the end of the experiment are presented in Table 4.8. Fish from Diet 2A had significantly ($p < 0.05$) the highest (79.80%) moisture and lowest lipid content (2.96%). There were no significant differences in carcass moisture contents of fish fed diets 2A, 4A, 4B, 6A and 6B. In general, fish from diets with higher levels of plant protein inclusion had higher moisture contents and lower lipid contents.

The final carcass protein levels ranged from 14.25% to 14.84%. There were no significant ($p > 0.05$) differences between the carcass protein contents of fish fed diets 3A and 3B but these values were significantly ($p < 0.05$) higher than for the rest of the diets. The carcass lipid contents in this experiment were comparatively higher than in Experiment 1. There were no significant ($p > 0.05$) differences between carcass lipid contents of fish fed diets containing 50% plant protein but these

values were significantly ($p < 0.05$) lower than for the rest of the diets.

4.3.7. General Health and Histological Examination

At the end of the experiment, no abnormalities or physical deformities were observed. Histological examination of the various organs such as gills, liver, muscle, kidney and intestine of fish fed different diets revealed no significant changes except for the diets containing 50% mustard oilcake protein (Diets 2A and 2B).

Liver histology of fish from Diets 2A and 2B revealed changes similar to those found for Diet 3 in Experiment 1 (Section 3.3.4). These changes included a higher level of intracellular lipid deposition compared to fine and evenly distributed granular cytoplasm noted in the hepatocyst of fish fed the Control diet. Histological examination of thyroid tissue of fish from Diets 2A and 2B (50% mustard protein) showed similar changes to those found in fish fed Diet 3(50% mustard) in Experiment 1 (Section 3.3.4).

4.4. DISCUSSION

Growth performance of carp showed improvement with essential amino acid (EAA) supplementation. Growth performance improvement was more pronounced in fish fed diets supplemented with all deficient EAA than in fish fed diets supplementing with the first limiting EAA. The improvements in protein utilization in diets supplemented with EAA in this experiment

confirm similar observations in poultry (Dean and Scott, 1965; Velu et al., 1971; Wethli et al., 1975) and pigs (Kabayashi et al., 1980; Keith and Bell, 1985). Mokady (1980) feeding algal protein to rats demonstrated that growth and protein efficiency could be improved substantially by methionine supplementation.

The growth improvements due to single or multipe EAA supplementation observed in the present study are in good agreement with the findings of various workers in numerous species including rainbow trout (Rumsey and Ketola, 1975; Dabrowska and Wojno, 1977); Atlantic salmon (Bergström, 1979; Ketola, 1982); Coho salmon (Spinelli et al., 1979; Mahnken, 1980; Ogata et al., 1983); Channel catfish (Robinson et al., 1980; Murai et al., 1982a) and common carp (Viola et al., 1982; Murai et al., 1982b, 1986).

Even though supplementation of crystalline amino acid did improve growth performance, these dietary groups did not produce growth equivalent to that of fish fed the fish meal based Control diet. The superiority of fish meal in this respect may be attributable to the absence of any known anti-nutritional factors and to the generally higher availability of the amino acids in fish meal protein (Abel et al., 1984).

EAA supplementation to diets containing 25% linseed protein (Diets 3A and 3B) significantly improved the growth but the performance was still significantly ($p < 0.05$) lower than that of the Control. Weight gains were about 90% (Diet 3A) and 96% (3B) respectively of the weight gain attained by fish fed the Control diet. The growth performance of fish

fed Diet 3B (supplemented with all deficient EAA) was significantly ($p < 0.05$) higher than that of fish fed Diet 3A (supplemented with first limiting EAA, lysine). Diet 3A was slightly deficient in methionine, phenylalanine and threonine as can be seen from the chemical scores (Table 4.4). The significantly better growth of fish fed Diet 3B was probably as a result of overall improved EAA balance (Table 4.4).

Appler (1984) found similarly improved growth performance of Tilapia zillii and Oreochromis niloticus fed diets supplemented with EAA. The growth performance improvement was more pronounced when algal diets were supplemented with histidine and methionine in combination, than with single EAA supplementation. Similar beneficial effects of multiple EAA supplementation have also been reported with trout. Fordiani and Ketola (1980) showed that addition of methionine or five EAA in combination tended to improve growth of trout fed raw soybean meal. With heated soybean meal growth was not improved by supplemental methionine alone but was significantly improved by the mixture of five EAA.

Contrary to the beneficial effects of EAA supplementation reported above some workers have shown that supplemental crystalline amino acids are less efficiently used by carp than amino acids in intact proteins (Aoe et al., 1970; Nose et al., 1974). Murai et al (1981) reported that carp utilized crystalline amino acids coated with casein more efficiently than those uncoated.

Possible explanations for this might be that coating of amino acids with casein minimized variation in absorption rate of individual amino acids

(by slowing down absorption of certain amino acids). This may result in relatively simultaneous presentation of amino acids to the tissues for optimal protein synthesis.

Unlike glycogen or fat synthesis, simultaneous presentation of all amino acids is essential for protein synthesis. Thus, not only the rapid absorption rate but also amino acid imbalance in the tissues, resulting from variation in the absorption rate of amino acids, may be responsible for poor utilization of crystalline amino acids by carp (Murai et al., 1981).

In the present study protein utilization (PER) was insignificantly different ($p > 0.05$) in Diets 3A, 3B (25% linseed protein) and the Control which were all significantly ($p < 0.05$) higher than for the rest of the diets. Supplementation of EAA to Diets 3A and 3B (25% linseed protein) improved PER compared to that obtained with diets (25% linseed protein) in Experiment 1 (Table 3.9). Although most satisfactory results were obtained with Diets 3A and 3B (25% linseed protein) the growth performances obtained were not equivalent to that of the Control. This is probably as a consequence of the anti-nutritional factors (HCN and phytic acid) present in linseed meal (Table 4.3).

Diets containing 50% mustard protein (Diets 2A and 2B) supplemented with either methionine as the first limiting EAA or multiple EAA (methionine, phenylalanine, lysine and threonine) produced improved growth performance compared to that of Diet 3 (50% mustard) in Experiment 1 (Table 3.9). In the present experiment, Diet 2B produced significantly ($p < 0.05$) higher

weight gains than Diet 2A but there were no significant differences in PER.

Although Diet 2A was slightly deficient in lysine, phenylalanine and threonine, the addition of methionine may have been the major factor responsible for the improved performance of fish fed Diets 2A compared to Diet 3 (50% mustard protein) in Experiment 1 (Table 3.9).

Murai et al. (1982b) found similar improvements in weight gain of carp fed diets containing soybean meal supplemented with methionine. In another investigation Murai et al. (1986) found almost three times higher free methionine in plasma of carp fed diets with methionine and other EAA than those fed unsupplemented diets. This is a positive indication that methionine supplementation improves the utilization of feed by carp. The importance of methionine supplementation within plant protein based fish feed and its beneficial effect on growth in salmonids has been reported by Poston et al. (1977). Pantha (1982) and Shiau et al. (1987) also reported that the supplementation of rations containing soybean with D-L-methionine is effective in increasing the nutritive value of soybean to tilapias.

In the present experiment both diets 2A and 2B contained 3.3 mg/g of allyl isothiocyanate and 1.14% tannins (Table 4.3). The reduced growth performance and feed utilization of fish fed these diets compared to the Control and Diets 3A and 3B could be attributed to the presence of these anti-nutritional factors.

Supplementation with lysine improved growth performances in all dietary groups. Chiu et al. (1986) also found more than 70% improvement in growth of milkfish fry fed a corn gluten meal based diet supplemented with the limiting amino acid lysine. Corn gluten meal also supported growth equal to that obtained with herring meal in trout when the diet was supplemented with certain amino acids, mainly lysine (Ketola, 1982). Keith and Bell (1985) also reported the beneficial effect of lysine supplementation in mustard meal for swine.

In the present trial fish fed Diet 5A (25% sesame protein) supplemented with lysine produced significantly ($p < 0.05$) lower growth performance and feed utilization than fish fed Diets 3A and 3B (25% linseed protein). The growth performance was also lower than for Diet 4 (25% linseed) in Experiment 1 (Table 3.9). However, Diet 5A contained less phytic acid (0.66%) than Diets 3A and 3B (0.78%). Apart from the possibly deleterious effect of phytic acid, the lower growth performance of fish fed Diet 5A could possibly be related to poor palatability and lower digestibility of the diet. For example, Diet 5A had an apparent protein digestibility of 79.76% whereas Diets 3A and 3B had protein digestibilities of 84.95% and 83.48% respectively.

All the diets containing 50% plant protein (Diets 2A, 2B, 4A, 4B, 6A and 6B) supplemented either with first limiting or multiple EAA, produced improved growth performance and food utilization compared to that of Experiment 1 (Table 3.9). But growth performance of fish fed these diets were still significantly ($p < 0.05$) lower than for fish fed Diets 1, 3A, 3B and 5A. These diets (2A, 2B, 4A, 4B, 6A and 6B) contained higher

levels of various anti-nutritional factors such as allyl isothiocyanate, phytic acid, HCN and tannins (Table 4.3). The observed poor growth performance of fish fed these diets could be attributed to the above mentioned anti-nutritional factors. The deleterious effects of phytic acid, HCN, allyl isothiocyanate and tannins on fish and other animals have been discussed in detail in Experiment 1 (Section 3.4).

On the basis of apparent nutrient digestibility coefficients determined, there was a progressive decrease in dry matter and apparent protein digestibility with the increasing plant protein substitution. Compared to Experiment 1 (Table 3.10) the apparent protein digestibility in the present experiment did not change very much with EAA supplementation of the diets. However, most of the diets containing plant proteins supplemented with EAA produced slightly higher protein digestibilities whereas the Control diet produced slightly lower digestibility. The effects various factors on nutrient digestibility have been discussed in detail in Experiment 1 (Section 3.4).

Changes in the body composition reflected those of growth and feed efficiency of respective dietary treatments. However, carcass moisture contents in all dietary groups were comparatively higher than for Experiment 1 (Table 3.11). The incorporation of various plant sources into the diets of fish has often resulted in a hitherto unexplained reduction in carcass lipid content of fish maintained on these diets. The reduction of carcass lipid and inverse relationship between carcass moisture and lipid as reported by other workers has been discussed in detail in Experiment 1 (Section 3.4).

The histological examination of various tissues at the end of the experiment showed marked changes in liver and thyroid tissues of the fish fed Diets 2A and 2B (50% mustard protein) similar to that observed in fish fed Diet 3 (50% mustard protein) in Experiment 1. The extent of these changes and the possible factors responsible for these changes have been discussed in Experiment 1 (Section 3.4).

The results of the present study demonstrated that carp can utilize crystalline amino acids and that supplementation with EAA improved the nutritive value of the diets containing plant proteins. However, the growth was still not equivalent to that of the fish meal based Control diet which may be attributed to the presence of various anti-nutritional factors in the selected plant proteins.

CHAPTER 5 : EXPERIMENT 3

Studies on the protein, energy and amino acid digestibility of fish meal, mustard oilcake, linseed and sesame meal for common carp (C. carpio L)

5.1 INTRODUCTION

A feedstuff may appear from its chemical composition to be an excellent source of nutrients but will be of little actual value unless it can be digested and absorbed. Knowledge of nutrient digestibility of the various feedstuffs used in formulating fish diets is desirable so that effective substitution of one ingredient for another may be achieved. Such substitution can be important in replacing fish meal protein and in the development of least-cost diets. Along with chemical analysis, digestibility determination will allow a more thorough evaluation of the performance of a particular protein source in a complete diet for fish (Plakas and Katayama, 1981).

Digestibility data can be obtained by two methods. The direct method consists of quantitatively collecting the faeces emitted that correspond to one or several meals (Post et al., 1965; Ogino et al., 1973). The direct method has the disadvantage of the need for special equipment, the necessity for force feeding and the consequent physiological stress caused by confinement (De La Noue and Choubert, 1986). The indirect method has been developed to overcome the problem of quantitative faecal collection using an inert marker (e.g. chromic oxide) incorporated into feed and analysing the marker both in feed and in the faeces.

Researchers have used various inert markers/indicators in digestibility studies with fish. To be an effective indicator a substance must be indigestible and unabsorbable, remain homogenously mixed with digesta during passage through the gut and having no effect on the digestive

metabolism of the target animal. Indigenous dietary components used as inert markers in digestibility studies in fish are silica (Hickling, 1966); cellulose (Buddington, 1979); hydrolysis resistant organic matter (Buddington, 1980; De Silva and Perera, 1983); hydrolysis resistant ash (Bowen, 1981; De Silva and Perera, 1983) and crude fibre (Tacon et al., 1983b; De Silva and Perera, 1983).

At present, chromic oxide (Cr_2O_3) is the most commonly used external dietary marker added to diets for estimation of nutrient digestibility of terrestrial animals (McDonald et al., 1981) and fish (Austreng, 1978; Tacon and Rodrigues, 1984; Wilson and Poe, 1985; Law, 1986).

Despite the fact that the chromic oxide method is convenient and has been widely used for studies on single ingredients, or combinations of ingredients in formulated fish feeds, faecal collection methods pose serious technical problems to the accuracy of reported results for both direct and indirect methods (Nose, 1967; Smith and Lovell, 1973).

As a result various techniques for faecal collection have been proposed and used: abdominal pressure (Nose, 1967); anal suction (Windell et al., 1978a); dissection (Hasting, 1969; Austreng, 1978; Wilson et al., 1981); metabolic chambers (Smith, 1971; Schmitz et al., 1983); settling of faeces (Cho and Slinger, 1979; Law, 1984) and continuous filtration of effluent water from fish tanks (Choubert et al., 1979, 1982).

These techniques suffer from a variety of deficiencies. If the samples of faeces are taken by stripping, there is a danger of interfering with

digestive processes in the hind gut which may lead to underestimation of apparent nutrient digestibility as may body fluid contamination (Cho and Slinger, 1979). However, faeces collected after being voided into water may suffer considerable leaching leading to an overestimation of digestibility (Windell et al., 1978a; Choubert et al., 1979).

In order to collect faeces from small experimental fish in the present study a specially designed tank system was used. Fish meal, mustard oilcake, linseed and sesame meals were selected as dietary protein sources on the basis of their availability and use in carp feeds in Bangladesh.

Lovell (1977) indicated that nutrient digestibility among feedstuffs varied considerably for different warm water fishes. However, most animal protein sources appear to be highly digested by fish. Some of the variability that has been reported results from the manner in which the feedstuffs were processed prior to being evaluated (Atack et al., 1979). The digestibility of plant protein, however, is largely determined by the amount of crude fibre in the respective feed (Kirchgessner et al., 1986).

Since the nutritive value of protein is influenced by physiological availability of its constituent amino acids, levels of amino acids determined chemically may not adequately reflect biological availability. Accurate information on the bioavailability of amino acids potentially has significant economic value permitting the formulation of diets that satisfy amino acid requirements without providing unnecessary excesses (Austic, 1983).

As pointed out by several authors, true digestibility is preferable to apparent digestibility to some extent because of difficulties in obtaining a uniform intake of all amino acids (Skrede, 1979). True amino acid availabilities determined by the faecal analysis methods are derived from their apparent availabilities by correcting the latter estimates for metabolic faecal amino acid levels. The metabolic faecal amino acid excretion is usually determined from faeces of animals fed protein-free diets.

Very few potentially useful ingredients can be used as the sole source of dietary protein. Most digestibility values for common fish feed ingredients have been determined by comparison of the digestibilities of a reference and test diet. The test diet being a mixture of reference diet and test ingredient (Smith et al., 1980; Cho et al., 1982; Wilson and Poe, 1985; Hilton and Slinger, 1986). The use of a reference diet assumes that there are no interactions between the components of the diets during digestion (Cho et al., 1982).

In contrast, Wilson et al. (1981) determined apparent and true amino acid availability values for various feed ingredients for channel catfish using each ingredient as a single source of protein. The results seem to indicate that valid estimates of amino acid digestibility are required for precise evaluation of protein sources and for economic formulation of diets.

Although several reports have appeared dealing with nutrient digestibility in various fishes, there is no published information on the amino acid availability of feed ingredients for carp. In view of this, the protein, energy and amino acid digestibilities of fish meal, mustard oilcake, linseed and sesame meals as single sources of protein in carp diets were determined in this study.

5.2 MATERIALS AND METHODS

5.2.1. Experimental System and Animals

The experimental system used was the same faeces collection system used in Experiment 1 (Section 3.2.1). The detailed design of the system is described in Section 2.1.2. The source of experimental fish and their quarantine procedures were as for Experiment 1 (Section 2.2.1). The average weight of the fish used in this experiment ranged between 6.6g and 6.9g.

5.2.2. Analytical Techniques

Proximate analyses of the ingredients, diets and faecal samples were carried out following the methods described in Section 2.3.1. The amino acid contents of the ingredients, diets and faecal samples were anlysed according to the methods described in Section 2.4.1. The essential amino acid tryptophan was not quantified due to its destruction during acid hydrolysis.

5.2.3. Diet Formulation and Preparation

One protein-free and four semi-purified diets were formulated using the following ingredients as single sources of protein:

1. Fish meal (Herring Meal) - Origin UK
2. Sesame (Sesamum indicum) - Origin India
3. Linseed (Linum usitatissimum) - expeller type; Origin Bangladesh
4. Indian mustard (Brassica juncea) - expeller type; Origin Bangladesh

The fish meal and sesame meal used in this experiment were from the same batch as used in Experiment 1 (Section 3.2.3). Linseed and mustard oilcake were imported directly from Bangladesh. All the ingredients were analysed for proximate composition the results of which are presented in Table 5.1. The amino acid composition of the test ingredients is presented in Table 5.2

All the test protein sources were finely ground to pass through a 790µm sieve to obtain a homogenous mixture. All diets, except the protein-free diet, were formulated to contain 30% protein contributed by a single protein source (Table 5.3). Because of the comparatively low protein contents in linseed and mustard oilcake, it was not possible to formulate diets containing more than 30% protein.

A protein-free diet was formulated to study the true digestibility of protein and amino acids. Dextrin and carboxymethyl cellulose were used

TABLE 5.1

Proximate composition of the dietary ingredients used in Experiment 3 (% dry matter basis)

Components	INGREDIENTS			
	Fish meal	Sesame meal	Linseed meal	Mustard oilcake
Dry matter	92.75	91.83	90.59	95.70
Crude protein	76.40	43.09	34.04	35.67
Crude lipid	9.09	7.67	5.80	8.60
Crude fibre	0.51	6.70	9.25	11.68
Ash	11.73	13.65	11.86	11.32
NFE[1]	2.27	28.89	39.05	32.73

[1] Nitrogen free extractives calculated as

100 - (Moisture+Crude protein+Crude fibre+Lipid+Ash)

TABLE 5.2

Amino acid composition (% dry matter) of the dietary protein sources used in Experiment 3

Amino Acids	Fish meal	Sesame meal	Linseed meal	Mustard oilcake
Aspartic acid	7.33	4.07	3.26	2.44
Threonine	3.16	1.61	1.24	1.47
Serine	2.63	1.88	1.43	1.30
Glutamic acid	10.47	9.04	6.13	6.04
Proline	3.37	1.65	1.46	2.11
Glycine	4.43	2.44	2.11	1.73
Alanine	4.66	2.16	1.61	1.48
Cystine	0.81	0.72	0.37	0.48
Valine	3.26	2.08	1.75	1.66
Methionine	2.14	0.76	0.29	0.69
Isoleucine	2.85	1.57	1.41	1.33
Leucine	5.15	2.84	1.93	2.23
Tyrosine	2.29	1.63	0.64	0.70
Phenylalanine	2.65	2.05	1.53	1.63
Histidine	*	1.08	0.96	0.89
Lysine	5.15	1.36	1.28	1.77
Arginine	4.48	5.05	2.23	2.20

* No data as peak not differentiated in the chromatogram

TABLE 5.3

Composition (%) of the experimental diets used in Experiment 3

Ingredients	DIETS				
	Protein-free	Fish meal	Sesame	Linseed	Mustard
Fish meal	-	39.27	-	-	-
Sesame meal	-	-	69.62	-	-
Linseed meal	-	-	-	88.13	-
Mustard oilcake	-	-	-	-	84.10
Cod liver oil	5.00	1.43	4.66	3.87	2.77
Soybean oil	5.00	5.00	-	-	-
Mineral premix[1]	4.00	4.00	4.00	4.00	4.00
Vitamin premix[1]	2.00	2.00	2.00	2.00	2.00
Binder (CMC)[2]	2.00	2.00	2.00	1.50	2.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Dextrin	26.00	25.00	5.50	-	2.00
α-cellulose	55.50	20.80	11.72	-	2.63
TOTAL	100.00	100.00	100.00	100.00	100.00

[1] For the composition of mineral and vitamin premixes see Tables 2.3 and 2.4 respectively

[2] Sodium carboxymethyl cellulose (high viscosity)

as carbohydrate source and binder respectively. α-cellulose was used as a bulking agent. The vitamin and mineral premixes were added at 2% and 4% respectively. Chromic oxide (0.5%) was added as a digestibility indicator. The dietary ingredients were well mixed and diets were prepared on a Hobart mixer (Section 2.2.5).

5.2.4 General Experimental Procedure

There were three replicates for each treatment with 12 fish in each replicate. The fish were acclimated to the experimental system for seven days before the start of the experiment. During the acclimation period fish were fed trout pellets (Ewos Baker's Omega No.3, 49% protein). A summary of the methodology used in this experiment is given in Table 5.4.

5.2.5. Feeding Rate

Fish were fed twice daily (09.00 hours and 17.00 hours) at 6% of their body weight per day. Because of the variation between the moisture contents of fish meal diet and other diets, feeding rate was calculated on a dry weight basis. The quantity of food fed per day was adjusted after each weekly weighing and fed for the subsequent week.

5.2.6. Faeces Collection

Faeces collection started four days after transfer to the experimental diets to allow evacuation of all previously ingested material. Faeces were collected separately for each replicate twice daily - morning and evening - for three weeks. The collection procedure was similar to that of Experiment 1 (Section 3.2.5). Collected faeces were dried in an oven

TABLE 5.4

A summary of the methodology used to study the digestibility of experimental diets

Fish	Cyrpinus carpio mean weight 6.69 ± 0.12g
Duration of Experiment	3 weeks
Treatments	Digestibility of protein, energy and amino acids in Fish meal, Sesame, Linseed and Mustard oilcake used as a single protein source in carp diet
No. of Treatments	5 (including protein-free diet)
Replication	3/treatment
Water Temperature	27° ± 1°C
Stocking Density	12/tank
Water Flow Rate	1 litre/minute
Feeding Rate	Twice daily at 6% of body weight
Faecal Collection	Collected twice daily for three weeks dried at 60°C in an oven and kept in airtight container for subsequent chemical analysis
Physicochemical characteristics monitored	Temperature, pH, dissolved oxygen and total ammonia

at 60°C and kept in air-tight containers for subsequent chemical analysis.

5.2.7. Analysis of the Experimental Data

Percent apparent digestibility of protein, energy and amino acids was calculated using the formula of Maynard and Loosli (1969) (Section 2.8.1.). The true digestibilities of protein and amino acids were calculated by correcting the apparent protein and amino acid data for metabolic faecal loss (MFL). The metabolic faecal protein and amino acids were determined by feeding a protein-free diet. Metabolic faecal losses were then expressed as gram per 100g diet. The true digestibilities of protein and amino acids were calculated using the formula of Kim (1974) as follows:

$$100 \times \left[\frac{\frac{\%\ \text{Nutrient in Feed}}{\%\ Cr_2O_3\ \text{in Feed}} - \left(\frac{\%\ \text{Nutrient in Faeces}}{\%\ Cr_2O_3\ \text{in Faeces}} - \frac{\text{gMFL/100g feed}}{\%\ Cr_2O_3\ \text{in Feed}} \right)}{\frac{\%\ \text{Nutrient in Feed}}{\%\ Cr_2O_3\ \text{in Feed}}} \right]$$

Where, MFL can be metabolic faecal nitrogen or amino acids.

5.2.8. Statistical Analysis

Differences in digestibility coefficients were tested for significance ($p < 0.05$) by Duncan's Multiple Range Test (Duncan, 1955). Standard error (±S.E.) of treatment means were calculated from the residual mean squares in the analysis of variance.

5.3 RESULTS

5.3.1. Analysed Composition of the Diets

The proximate and amino acid compositions of the experimental diets are shown in Tables 5.5 and 5.6 respectively. Analysed protein and lipid contents of experimental diets showed little variation. However, the ash and crude fibre contents of the diets varied considerably (Table 5.5). Energy contents of the diets ranged between 3.95 and 4.24 Kcal/g. The protein-free diet contained 0.22% crude protein possibly contributed by non-protein nitrogen fractions of the ingredients.

5.3.2. Faecal Amino Acid Composition

The faecal amino acid patterns of carp fed the experimental diets are presented in Table 5.7. All the amino acid contents, except tyrosine, in the faeces of fish fed the fish meal diet were significantly ($p < 0.05$) higher than for the rest of the diets. On the other hand, faecal amino acid contents for fish fed the sesame diet were significantly ($p < 0.05$) lower than for fish fed the mustard and linseed diets (except arginine and histidine). However, there were no significant ($p > 0.05$) differences between threonine, serine, glutamic acid, alanine, cystine, valine, methionine, tyrosine and arginine contents in the faeces of fish fed the linseed or mustard diets (Table 5.7).

The faecal amino acid pattern of carp fed the protein-free diet is shown in Table 5.8. For comparison, the metabolic faecal amino acid patterns of channel catfish and rainbow trout fed protein-free diets (as found by other workers) are presented in Table 5.8.

TABLE 5.5

Analysed proximate composition of the experimental diets used in Experiment 3 (% dry matter basis unless otherwise stated)

Components	DIETS				
	Protein-free	Fish meal	Sesame	Linseed	Mustard
Dry matter	94.02	96.03	92.12	92.18	92.28
Crude protein	0.22	31.60	30.45	29.22	30.12
Crude lipid	9.56	10.20	9.72	9.05	10.12
Ash	4.70	8.65	13.43	13.52	13.74
Crude fibre	50.56	18.48	15.32	8.25	12.14
NFE[1]	34.96	31.07	31.08	39.96	33.88
Chromic Oxide	0.50	0.46	0.49	0.48	0.51
Energy (Kcal/g)	-	4.10	3.95	4.15	4.24

[1] Nitrogen free extractives calculated as
100 - (Moisture+Crude protein+Crude lipid+Ash+Crude fibre)

TABLE 5.6

Analysed amino acid composition of the experimental diets used in Experiment 3 (% dry matter basis)

Amino acids	DIETS			
	Fish meal	Sesame meal	Linseed meal	Mustard oilcake
Aspartic acid	3.32	2.93	2.93	2.29
Threonine	1.40	1.25	1.23	1.39
Serine	1.23	1.34	1.40	1.26
Glutamic acid	4.13	6.61	5.80	5.38
Proline	1.39	1.20	1.27	2.02
Glycine	1.94	1.40	1.88	1.68
Alanine	1.90	1.85	1.61	1.46
Cystine	0.32	0.52	0.44	0.48
Valine	1.72	1.45	1.75	1.64
Methionine	0.85	0.49	0.31	0.27
Isoleucine	1.56	1.30	1.50	1.32
Leucine	2.64	2.14	1.95	2.22
Tyrosine	0.90	0.97	0.72	0.67
Phenylalanine	1.44	1.43	1.52	1.24
Histidine	*	0.80	0.62	0.74
Lysine	2.09	1.12	1.24	1.75
Arginine	2.10	2.67	2.16	2.19

* No data as peak not differentiated in the chromatogram

TABLE 5.7

Faecal amino acid pattern of carp fed the experimental diets

(% dry matter basis)

Amino acids	DIETS				
	Fish meal	Sesame	Linseed	Mustard	± S.E.[2]
Aspartic acid	0.89[a1]	0.55[d]	0.77[b]	0.63[c]	0.02
Threonine	0.39[a]	0.25[c]	0.33[b]	0.37[ab]	0.01
Serine	0.34[a]	0.26[b]	0.34[a]	0.33[a]	0.01
Glutamic acid	0.96[b]	1.00[b]	1.38[a]	1.38[a]	0.02
Proline	0.39[b]	0.27[d]	0.32[b]	0.57[c]	0.01
Glycine	0.54[a]	0.31[d]	0.47[b]	0.42[c]	0.01
Alanine	0.47[a]	0.34[c]	0.40[b]	0.40[b]	0.01
Cystine	0.09[b]	0.08[b]	0.13[a]	0.13[a]	0.01
Valine	0.44[a]	0.30[b]	0.44[a]	0.43[a]	0.01
Methionine	0.13[a]	0.10[b]	0.08[c]	0.08[c]	0.004
Isoleucine	0.42[a]	0.27[d]	0.39[b]	0.35[c]	0.01
Leucine	0.61[a]	0.41[c]	0.50[b]	0.59[a]	0.02
Tyrosine	0.22[a]	0.21[a]	0.19[a]	0.18[a]	0.01
Phenylalanine	0.45[a]	0.31[c]	0.40[b]	0.33[c]	0.01
Histidine	*	0.14[b]	0.15[b]	0.19[a]	0.01
Lysine	0.52[a]	0.25[c]	0.36[b]	0.50[a]	0.01
Arginine	0.99[a]	0.63[b]	0.60[b]	0.64[b]	0.02

[1] Figures in the same row having the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

* No data as peak not differentiated in the chromatogram

TABLE 5.8

Faecal amino acid pattern of carp fed a protein-free diet as compared to those of channel catfish and rainbow trout

Amino acids	CARP		CHANNEL CATFISH		RAINBOW TROUT[2]
	mg/100g diet	g/100g protein	mg/100g diet	g/100g protein	g/100g protein
Aspartic acid	109±8	8.9	100±11	11.7	11.5
Threonine	65±3	5.3	56±6	6.6	9.5
Serine	58±6	4.8	82±9	9.6	5.3
Glutamic acid	113±9	9.3	89±6	10.4	11.4
Proline	42±2	3.4	50±3	5.9	7.2
Glycine	64±5	5.2	53±3	6.2	5.8
Alanine	51±4	4.2	38±3	4.5	5.1
Cystine	11±2	0.9		-	-
Valine	62±7	5.1	40±4	4.7	5.3
Methionine	31±5	2.5	16±2	1.9	-
Isoleucine	43±5	3.5	33±2	3.9	4.4
Leucine	65±10	5.3	56±6	6.6	6.6
Tyrosine	38±2	3.1	42±5	4.9	3.5
Phenylalanine	49±5	4.0	38±3	4.5	4.4
Histidine	*	*	42±5	4.9	-
Lysine	69±10	5.7	80±14	9.4	5.5
Arginine	121±7	9.9	38±3	4.5	4.0

[1] Data from Wilson et al. (1981)

[2] Data from Skrede et al. (1980)

[3] Mean of three replicates ± standard deviation

* No data as peak not differentiated in the chromatogram

5.3.3. Protein and Energy Digestibility

Apparent protein digestibility (APD) and true protein digestibility (TPD) for the experimental diets were determined as described in Sections 2.8.1 and 5.2.7 respectively. The fish meal diet showed significantly ($p < 0.05$) the highest (88.9%) and the sesame diet the lowest (78.9%) APDs. However, there were no significant ($p > 0.05$) differences between the APDs of the linseed and mustard diets and these were intermediate between the fish meal and sesame diets. Similar trends were observed in the TPDs of the experimental diets, TPDs ranged from 82.9% to 92.4% (Table 5.10).

Significantly ($p < 0.05$) the highest apparent energy digestibility (AED) was found for the fish meal diet. There were no significant ($p > 0.05$) differences in AED between the linseed, mustard and sesame diets (Table 5.9).

5.3.4. Amino Acid Digestibility

Apparent amino acid digestibilities (AAAD) were calculated according to the formula given in Section 2.8.1 and results are presented in Table 5.9. The average AAAD values for the various diets ranged from 82.4% to 90.5%. All AAAD values for the fish meal diet were significantly ($p < 0.05$) higher than for the rest of the diets. For most amino acids the AAAD values of the sesame diet were significantly ($p < 0.05$) lower than for the mustard and linseed diets (the exceptions being glutamic acid, cystine, methionine and arginine). However, there were no significant ($p > 0.05$) differences between AAAD values of linseed and mustard diets

TABLE 5.9

Apparent protein, energy and amino acid digestibility (%) of the experimental diets

Components	DIETS				
	Fish meal	Sesame	Linseed	Mustard	± S.E.[2]
Protein	88.9[a1]	78.9[c]	85.8[b]	85.3[b]	0.40
Energy	80.1[a]	69.9[b]	72.1[b]	71.4[b]	0.71
Aspartic acid	90.4[a]	83.5[d]	86.7[b]	85.4[c]	0.38
Threonine	90.1[a]	82.0[c]	85.5[b]	85.9[b]	0.80
Serine	90.6[a]	82.5[c]	85.8[b]	86.2[b]	0.66
Glutamic acid	91.8[a]	86.5[b]	86.3[b]	86.4[b]	0.38
Proline	90.2[a]	79.8[c]	85.8[b]	85.4[b]	0.49
Glycine	90.1[a]	80.2[c]	86.2[b]	86.6[b]	0.41
Alanine	91.2[a]	83.5[c]	86.1[b]	86.4[b]	0.50
Cystine	90.4[a]	85.8[b]	84.0[b]	85.6[b]	0.78
Valine	90.7[a]	81.5[c]	85.9[b]	85.9[b]	0.56
Methionine	94.6[a]	82.5[b]	85.0[b]	84.9[b]	0.80
Isoleucine	90.4[a]	82.4[c]	85.6[b]	86.1[b]	0.78
Leucine	91.8[a]	82.9[c]	85.8[b]	85.9[b]	0.64
Tyrosine	91.5[a]	80.5[c]	85.5[b]	85.5[b]	0.68
Phenylalanine	89.0[a]	81.0[c]	85.5[b]	86.0[b]	0.57
Histidine	*	84.1[b]	87.1[a]	86.6[ab]	0.78
Lysine	91.2[a]	80.5[c]	83.7[b]	84.8[b]	0.40
Arginine	83.3[a]	81.1[a]	84.5[a]	84.6[a]	1.43
AVERAGE AAAD	90.5	82.4	85.6	85.8	-

[1] Figures in the same row having the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of mean calculated from the residual mean square in the analysis of variance

* No data as peak not differentiated in the chromatogram

TABLE 5.10

True protein and amino acid digestibility (%) of the experimental diets

Components	DIETS				
	Fish meal	Sesame	Linseed	Mustard	± S.E.
Protein	92.4[a1]	82.9[c]	89.9[b]	89.3[b]	0.42
Aspartic acid	92.9[a]	86.2[c]	89.5[b]	88.9[b]	0.38
Threonine	92.9[a]	85.8[c]	88.3[b]	88.9[b]	0.64
Serine	92.8[a]	85.2[c]	88.9[b]	88.7[b]	1.01
Glutamic acid	94.0[a]	88.0[b]	88.2[b]	88.1[b]	0.24
Proline	91.6[a]	82.8[d]	87.7[b]	86.4[c]	0.31
Glycine	92.6[a]	85.2[c]	88.8[b]	89.8[b]	0.85
Alanine	92.8[a]	85.4[c]	88.2[b]	88.6[b]	0.48
Cystine	93.8[a]	87.9[b]	86.5[b]	88.0[b]	0.78
Valine	94.7[a]	86.6[c]	89.6[b]	89.8[b]	0.36
Methionine	98.1[a]	90.8[c]	95.1[b]	96.4[ab]	0.52
Isoleucine	92.3[a]	86.5[c]	87.6[bc]	89.0[b]	0.72
Leucine	93.7[a]	86.2[c]	88.4[b]	88.2[b]	0.51
Tyrosine	93.6[a]	82.0[c]	87.7[b]	88.3[b]	0.85
Phenylalanine	91.0[a]	83.1[c]	87.4[b]	88.4[b]	0.56
Histidine[3]	-	-	-	-	-
Lysine	93.6[a]	86.9[b]	87.8[b]	87.7[b]	1.10
Arginine	89.1[a]	83.5[b]	90.2[a]	90.1[a]	1.03
AVERAGE TAAD	93.1	85.7	88.7	89.1	-

[1] Figures in the same row having the same superscripts are not significantly ($p > 0.05$) different

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

[3] No data as peak not differentiated in the chromatogram

(except aspartic acid).

True amino acid digestibilities (TAAD) are presented in Table 5.10 and ranged from 85.7% to 93.1%. TAAD showed similar trends to AAAD. As for AAADs all TAADs (except arginine) for the fish meal diet were significantly ($p < 0.05$) higher than for the rest of the diets. There were no significant ($p > 0.05$) differences between TAADs of mustard and linseed diet (except proline). However, most of the TAAD values (except glutamic acid and lysine) for the sesame diet were significantly ($p < 0.05$) lower than for the mustard and linseed diets.

5.4 DISCUSSION

Of the ingredients tested in this study fish meal was the most digestible with an apparent protein digestibility (APD) of 88.9% (Table 5.9). This is in agreement with values reported for carp and other species by various authors (Table 5.11). According to NRC (1977) carp can digest 95% of the protein in fish meal however this value can decrease to 80%-85% depending on the origin and processing of the fish meal concerned (Ogino and Chen, 1973). Similarly, the crude protein digestibility of other animal protein sources such as blood meal, meat and bone meal and feather meal, is mainly determined by the processing methods employed. For example, blood meal may be damaged by heat so that its crude protein is only 60% digested by carp and possibly only 40% if the level in the feed mixture is high (Schwarz et al., 1986).

TABLE 5.11

Protein digestion coefficient of some commonly used feed ingredients for carp, channel catfish and rainbow trout

Ingredients	Carp	Channel catfish	Rainbow trout	References
Fish meal	83.5 (1) 88.8 (2) 80.3 (3) 95.0* (9) 90.2* (2)	85.0 (4) 86.0 (5)	86.7 (6) 84.0 (7) 91.0 (11)	(1) = Ufodike and Matty (1983) (2) = Kim (1974) (3) = Atack et al. (1979) (4) = Wilson and Poe (1985) (5) = Brown et al. (1985) (6) = Smith et al. (1980) (7) = Kitamikado et al. (1964) (8) = Inaba et al. (1963) (9) = NRC (1977) (10) = Hilton & Slinger (1986) (11) = Pfeffer (1982) (12) = Ogino and Chen (1973)
Soybean meal	86.8 (2) 83.7 (8) 95.5* (12)	85.0 (5) 97.0 (4)	80.0 (6) 86.4 (11)	
Petroyeast	96.6 (3) 86.8* (12)		91.6 (3)	
Rapeseed meal			76.4 (6) 63.8 (11)	
Meat and Bone meal		82.0 (5) 61.0 (4)		
Corn Gluten meal	91.2* (12)	92.0 (5)	93.0 (11)	
Cottonseed meal		83.0 (4)	80.3 (6)	
Peanut meal	85.0 (9)	86.0 (5)		
Linseed meal			76.7 (6)	
Poultry By-products		65.0 (5)		
Silkworm pupae meal	63.9 (2)			
Canola meal			87.0 (10)	

*True digestibility

True protein digestibility is independent of protein intake (Nose, 1967; Ogino and Chen, 1973; Rychly and Spanhof, 1979), hence, the coefficients obtained in this study can be compared with those of other studies employing different protein levels. The TPD of fish meal (92.4%) obtained here is in agreement with the reported value of 90.2% by Kim (1974) and 95% by NRC (1977) for carp. An important assumption in true digestibility calculation is that the endogenous or metabolic faecal protein production in all the experimental diets is the same as that in the protein-free diet. Possible sources of endogenous faecal protein includes:

1. enzymes secreted from various regions of the digestive system;

2. cells and cellular debris sloughed off from the mucosa or backflux of amino acids from fish enterocytes (Ferraris and Arhearn, 1984).

Both the mustard and linseed diets exhibited good APDs (85.8% and 85.3% respectively, Table 5.9). These are generally in agreement with the reported APD values of various oilseed meals as reported by other workers (Table 5.11). For example, soybean meal (86.8%) in carp (Kim, 1974); canola meal (87%) in rainbow trout (Hilton and Slinger, 1986); peanut meal (85%) in carp (NRC, 1977) and cottonseed meal (83%) in channel catfish (Wilson and Poe, 1985). Smith et al. (1980) reported a somewhat lower APD of 76.7% for linseed meal in rainbow trout.

The comparatively lower APDs obtained in Experiment 1 (Section 3.3.8) using mustard and linseed meal in combination with fish meal might have been due to interactions between feedstuffs in a diet. Kirchgessner et al. (1986) suggested that the digestibility of an individual feed might

be weakened by its feeding in a mixture with other components.

The sesame diet in the present study had a significantly ($p < 0.05$) lower APD (78.9%) than either the linseed (85.8%) or mustard diets (85.3%, Table 5.9). However, this value is comparable to the reported APDs of rapeseed meal (76.4%) and cottonseed meal (80.3%) in rainbow trout (Smith et al, 1980). One possible factor responsible for the lower APD of some sesame meal could be its higher phytic acid content (Gohl, 1981; ADCP, 1983). Spinelli et al. (1983) reported that as low as 0.5% dietary phytic acid resulted in lower protein digestibility in rainbow trout.

The TPDs of the linseed (89.9%), mustard (89.3%) and sesame diets (82.9%) in this study are comparable to the TPD of petroleum yeast (86.8%) and slightly lower than the TPD of soybean meal (95.5%) reported for carp (Ogino and Chen, 1973). From the results of this experiment, it is also evident that reducing the protein level from 40% to 30% in a diet did not reduce the digestibility of the protein. Jauncey (1982) also reported that in S. mossambicus juveniles the protein digestibility was not affected by the dietary protein concentration.

The apparent energy digestibility (AED) for fish meal in the present study (80.3%, Table 5.9) is higher than reported by some authors (74%, Windell et al., 1978b) for rainbow trout but lower than reported by others (95%, Smith et al., 1980; 91%, Cho et al., 1982) for the same species. It is similar to the value reported for grass carp (83%, Law, 1986). The AED for linseed (72.4%) found here agrees with the reported AED value for linseed (71%) in trout (Smith et al., 1980). AED values of

mustard, linseed and sesame diets in this study are slightly lower than reported values for canola meal (75%) and dehulled soybean meal (75%) for trout (Hilton and Slinger, 1986 and Cho et al., 1982, respectively); peanut meal (76%) for channel catfish (Wilson and Poe, 1985); and somewhat higher than for rapeseed meal (65%) and cottonseed meal (64%) but similar to soybean meal (70.8%) for trout (Smith et al., 1980).

The lower AED values of fish meal obtained here compared to that reported for trout could possibly be due to the higher crude fibre content (18.48%) in the present fish meal diet. The high crude fibre in the fish meal diet resulted from the higher α-cellulose inclusion in order to keep the protein, nitrogen free extractives (NFE) and energy contents at desired levels. Hilton et al. (1983) suggested that a higher dietary crude fibre content may accelerate the passage of digesta through the intestinal tract thus reducing the digestibility of energy and protein.

Viola et al. (1982), working with Cyprinus carpio, indicated that certain plant protein meals may have a reduced availability of energy and lysine to fish, which may account for the inability of plant protein to successfully replace fish meal.

One of the functions of absorbed amino acids is to replace that part of the body protein which inevitably lost by animals during normal activity. Part of these losses are metabolic faecal nitrogen, that proportion of faecal nitrogen which does not represent undigested nitrogenous components of the diets.

Studies comparing the amino acid composition of metabolic faecal protein in rats and cocks (Slump et al., 1977) have shown only minor differences between species. A comparison of metabolic faecal amino acids (expressed as g/100g protein) from carp, channel catfish (Wilson et al., 1981) and rainbow trout (Skrede et al., 1980) indicates a similar pattern (Table 5.8).

This similarity is probably due to the comparable amino acid composition of the various digestive enzymes found in the gastrointestinal tract of these animals (Wilson et al., 1981). The apparent differences in serine, proline, lysine, arginine (Table 5.8) may be due to either a difference in composition of the sloughed cell wall material or to a difference in intestinal microbial activity between these animals.

In the present study, the sum of faecal amino acid contents of the experimental diets varied between 78% and 97% of faecal crude protein. Kaushik and Luquet (1976) found, in faeces of trout, the sum of the amino acids measured corresponded to more than 85% of the faecal crude protein.

The average apparent amino acid digestibility (AAAD) value (90.5%, Table 5.9) of fish meal found here is slightly higher than the APD (88.9%) value. Vens-Cappell (1984) also found a similarly higher average AAAD value (87%) than APD value (84%) in trout with both extruded and pelleted diets. The average AAAD value of fish meal found here is higher than the reported value of 82.9% for channel catfish (Wilson et al., 1981); and 80.6% for chicks (Soares et al., 1971). Although carp fail to grow normally when fed diets containing amino acids as their sole nitrogen

source (Aoe et al., 1970, 1974; Nose, 1974), the AAAD value of a free amino acid test diet is reported to be as high as 86.4% (Plakas and Katayama, 1981). However, the higher average AAAD value obtained for the fish meal diet in comparison to those reported above is most likely to be due to differences in composition and processing of the fish meal under test.

The average AAAD value (82.4%) for the sesame diet was higher than its APD value (78.9%), however for linseed and mustard diets the average AAADs (85.6% and 85.8% respectively) were similar to their APD values (85.8% and 85.3% respectively). The average AAAD values of mustard, linseed and sesame meal generally agree with reported AAADs of other oilseed meals such as soybean meal (81%) and peanut meal (88.4%) in channel catfish (Wilson et al., 1981); and canola meal (84.7%) in trout (Hilton and Slinger, 1986). Amino acid availability depends on the digestibility coefficient of a protein and the rate of release of amino acids during digestion (Swaminathan, 1967). However, the variation in AAAD values reported above may be, in part, explained by the anatomical and physiological differences in the gastrointestinal tracts of carp, trout and channel catfish.

Methionine, tryptophan, arginine and lysine are likely amino acids to be adversely affected by the various processing techniques applied to oil-seed meals and fish diets (Leiner, 1980). The AAADs for lysine and arginine in mustard, linseed and sesame meal diets are comparatively lower than for the rest of the amino acids (Table 5.9). Viola et al. (1982) also indicated a low availability of lysine in soybean meal to

fish.

The TAAD values of all the ingredients in this study are higher than AAAD values although there was little difference (about 3%) observed between these values. Similarly small differences were observed by Wilson et al. (1981) between TAADs and AAADs of menhaden meal, soybean, cottonseed and peanut meal in channel catfish.

In summary, data presented here suggest a reasonable agreement between protein digestibility and average AAAD values. In general, the protein digestibility values were indicative of amino acid digestibility. However, because of variations within individual amino acid digestibilities in a feed ingredient, the use of specific amino acid digestibility value would allow more accurate and economic feed formulation. From the nutrient digestibility viewpoint it appears that carp may be able to utilize mustard, linseed and sesame meals efficiently as dietary protein sources.

CHAPTER 6 : DETOXIFICATION OF OILSEED MEALS

EXPERIMENT 4.1

Detoxification of mustard oilcake and evaluation of its nutritive value in the diet of common carp (C. carpio L)

6.1 INTRODUCTION

Many plants contain glucosides which, upon hydrolysis by endogenous enzymes, release substances which may be toxic (Liener, 1977). Examples of such glucosides are the goitrogens of rapeseed, mustard seed and cyanogens of linseed. These goitrogenic products are primarily isothiocyanates liberated from the plant tissue which act on their respective substrates when the seed is crushed and moistened.

In the case of mustard seed, the principal hydrolytic product of the glucoside, sinigrin, is allyl isothiocyanate which, unless removed from the meal, imparts an extremely pungent flavour and thus limits the amount that can be used for feeding purposes (Mustakas et al., 1963; Liener, 1977; Shah et al., 1977).

Suitable processing techniques or traditional methods of preparation often take advantage of the enzymatic release of toxic constituents in order to detoxify such foods. The destruction of these constituents may also be facilitated by aqueous treatment, heat treatment or autoclaving.

Inactivation of myrosinase has offered a feasible solution to the problems in processing and utilization of rapeseed meal (Maheshwari et al., 1981). Myrosinase starts to become inactivated at temperatures above 70°C, if the moisture content in rapeseed is 6% to 10%. Reynolds and Young (1964) demonstrated that cooking crushed rapeseed without addition of water at 80°C destroyed myrosinase activity. Appelqvist and Josefsson (1967) found, in laboratory studies, that the myrosinase in

seeds with 8% moisture was completely inactivated by heating at 90°C for 15 minutes.

Residual myrosinase activity in rapeseed meals has been destroyed by treating the meal with hot water at 90°C (Belzile et al., 1963) and steam autoclaving for 15 minutes at 1.2 kg/cm^2 pressure (Bell et al., 1963). Belzile et al. (1963) produced dried meals devoid of glucosinolates and their hydrolytic products.

Eapen et al. (1968) demonstrated that micro-wave heating of whole rapeseed at their original moisture contents of 5.2% and 6% for three minutes inactivated the enzyme but produced oil with a darker colour and meal with a burnt aroma. Rutkowski (1970) reported that temperatures of 120°C and above in industrial toasting trials destroyed more than 50% of the isothiocyanates and oxazolidinethione contents of rapeseed meal but resulted in about one-third decrease in the amount of soluble proteins. He also maintained that the more drastic the treatment applied, the greater will be the destruction of goitrogens. Bell et al. (1981) also reported that heat treatment alone resulted in significant reduction (about 50% of original) in glucosinolate content, especially of the allyl-type, in mustard seed (B. juncea).

In autoclaving, the pressure used has a marked effect on disappearance of isothiocyanate and oxazolidinethione in rapeseed meal. At higher pressure (1.2 kg/cm^2) about 75% of the original isothiocyanate and over 90% of the oxazolidinethione had disappeared (Bell and Belzile, 1965). Srivastava and Hill (1976) reported that heat treatment (dipping seeds in

boiling water) of two varieties of rapeseed (B. napus) produced improved weight gains and lower thyroid weights in rats who received these meals as sole sources of protein. Steaming of untreated meals similarly gave increased weight gains. Although destruction of the enzyme by moist heat treatment prevents the further breakdown of the thioglucosides in the meal, the possibility remains that the residual glucoside could be hydrolysed after ingestion of toxic end products by enzymes produced in the intestinal tract (Marangos and Hill, 1974).

Goering (1963) developed a process in which ground rapeseed was moistened with cold water to potentiate the enzyme or the rapeseed meal was made into slurry with six to eight parts of water. Myrosinase was added, if necessary, and digestion carried out at 45-55°C. The liberated volatile oils of mustard, isothiocyanates, were removed by steam stripping. Mustakas et al. (1963) also developed a similar method to produce oil, meal and allyl isothiocyanate from B. juncea, the residual meals contained only 0.004% allyl isothiocyanate. The advantage of this method is that allyl isothiocyanate is not only water soluble but volatile as well, so it can be removed by distillation after enzymatic hydrolysis of the glucoside.

Ballester et al. (1970) used two types of treatments, steaming and double water extraction, to detoxify rapeseed meal and reported that double water extraction (first 12h water extraction followed by second extraction at room temperature) gave the best results. It resulted in a reduction of 84% in oxazolidinethione and 77% in isothiocyanate and net protein utilization in rats increased from 40% to 69%. In contrast,

steaming and simple water extraction, however, reduced toxicity only slightly and improved nutritive value very little.

In a further study Ballester et al. (1973) reported that a 2h continuous water extraction procedure with stirring (Lixiviation process) completely removed the isothiocyanates and reduced the oxazolidinethione by 96%. The detoxified meal obtained by this process gave PERs and growth rates similar to casein diets in rats.

Mukharjee et al. (1976) also proposed a method of autolysis of ground rapeseed by action of heat (45°C), moisture (15%-40%) and myrosinase followed by defatting with hexane, for the production of low-glucosinolate rapeseed meals. Agren and Eklund (1972) prepared detoxified protein concentrate from rapeseed (B. napus) by hydraulic processing and reported that the weight gain and protein efficiency ratio in rat was equal to that obtained with a reference diet.

Shah et al. (1977) used various methods for detoxification of mustard seed cake and concluded that maximum detoxification of mustard seed can be accomplished by enzymatic or aqueous treatments. Feeding rats with aqueous extracted meal with pan dried supernatant suppressed the growth but aqueous extracted meal when supplemented with proteins coagulated, by steam passage, showed a significant increase in growth. Enzymatically detoxified meal, when supplemented with pan/spray dried supernatants produced comparatively lesser weight gain. Increased hair loss and mortality of rats in this study indicated that free allyl isothiocyanate, liberated during enzymatic detoxification, formed highly toxic substances

during pan drying, with carbohydrate and protein present in the supernatant (Shah et al., 1977).

Abel et al. (1984) treated full-fat soybeans thermally (118°C) or hydrothermally (90°-95°C), either gently (0.5 to 15 minutes) or intensely (2.5 or 30 minutes). It was observed that the trypsin inhibitor was sensitive to moist heat and was almost completely destroyed even in the hydrothermally gentle treatment. In feeding trials with carp, soybean with intensely thermal and hydrothermal treatments produced higher growth performances than for fish fed a commercially available feed.

Considering the poor growth performances of carp on diets containing mustard oilcake (MOC) in Experiments 1 (Table 3.9) and 2 (Table 4.6), an attempt was made to detoxify MOC using various processing techniques and to evaluate the nutritive value of detoxified MOC in the diet of common carp.

6.2 MATERIALS AND METHODS

6.2.1. Experimental System and Animals

The experimental systems used in this study are described earlier (Sections 2.1.1 and 2.1.2). The sources of experimental animals and their quarantine procedure are described in Section 2.2.1.

6.2.2. Analytical Techniques

The analytical techniques used were as described in Chapter 2.

6.2.3. Detoxification of Mustard Oilcake (MOC)

Mustard oilcake (B. juncea) was of expeller type and obtained from Bangladesh. Detoxification of MOC was accomplished by various treatments as follows:

1. Heat Treatment

The ground MOC was subjected to steam autoclaving for 1 hour at 1 Kg/cm^2 pressure in an autoclave. The meals were air dried at 40°C using an electric fan convector heater.

2. Aqueous Treatment

Aqueous extraction of MOC was carried out according to Ballester et al. (1970). MOC was soaked in five times its weight of water and kept at room temperature for 18 hours. After treatment, the oil-cakes were filtered and air dried at 40°C using an electric fan convector heater.

Treatment of Supernatant

The supernatant was steamed for 30 minutes to coagulate the protein present. The coagulated proteins were separated by centrifugation at 500 rpm for 15 minutes and air dried at 40°C using an electric fan convector heater.

3. Enzyme Treatment

MOC was suspended in water in a ratio of 1:3 and incubated at 60°C in an oven for 1 hour for enzymatic hydrolysis of glucosinolates. The free isothiocyanate was removed by steam distillation and the meal was filtered and air dried at 40°C using an electric fan

TABLE 6.1

Proximate composition of dietary ingredients used in Experiment 4.1

(% dry matter basis)

Components	INGREDIENTS						
	Fish meal	Untreated MOC (UM)	Heat treated MOC (HM)	Aqueous treated MOC (AM)	Aqueous treated MOC + soluble protein (AMP)	Enzyme treated MOC (EM)	Enzyme treated MOC + soluble protein (EMP)
Dry matter	92.23	92.92	92.98	92.75	93.14	91.92	92.08
Crude protein	76.95	36.97	36.70	34.86	35.95	34.81	35.92
Crude lipid	10.19	7.92	7.66	7.65	7.60	7.66	7.60
Ash	11.73	11.45	11.36	11.27	11.23	11.34	11.29
Crude fibre	0.55	10.63	10.64	10.65	10.64	10.64	10.63
NFE[1]	0.57	33.02	33.61	35.55	34.60	35.43	34.48

[1] Nitrogen free extractives calculated as

100 - (Moisture+Crude protein+Crude lipid+Ash+Crude fibre)

TABLE 6.2

Amino acid composition (% dry matter) of the dietary protein sources used in Experiment 4.1

Amino acids	Fish meal	Untreated MOC (UM)	Heat treated MOC (HM)	Aqueous treated MOC (AM)	Aqueous treated MOC + soluble protein (AMP)	Enzyme treated MOC (EM)	Enzyme treated MOC + soluble protein (EMP)
Arginine	5.07	2.06	2.04	2.00	2.01	2.09	2.07
Histidine	1.61	0.86	0.84	0.81	0.82	0.65	0.72
Isoleucine	3.28	1.29	1.30	1.22	1.24	1.26	1.25
Leucine	5.74	2.21	2.20	2.14	2.14	2.08	2.12
Lysine	5.48	1.65	1.60	1.55	1.61	1.65	1.64
Methionine	2.23	0.44	0.43	0.33	0.40	0.35	0.41
Cystine	0.84	0.48	0.49	0.43	0.45	0.41	0.46
Phenylalanine	2.81	1.38	1.38	1.30	1.36	1.29	1.27
Tyrosine	2.08	0.72	0.71	0.65	0.69	0.66	0.73
Threonine	3.18	1.55	1.54	1.49	1.50	1.46	1.51
Valine	4.00	1.68	1.61	1.56	1.58	1.53	1.57
Alanine	4.68	1.42	1.40	1.38	1.38	1.34	1.41
Aspartic acid	7.59	2.46	2.44	2.34	2.37	2.30	2.41
Glutamic acid	10.38	5.71	5.70	5.59	5.65	5.32	5.61
Glycine	4.48	1.58	1.57	1.52	1.52	1.49	1.52
Proline	3.49	2.19	2.20	2.10	2.16	2.12	2.18
Serine	2.60	1.26	1.26	1.23	1.23	1.20	1.31

TABLE 6.3

Effect of various processing treatments on available lysine, allyl isothiocyanate and tannin contents (% dry matter basis) of mustard oilcake

Components	Untreated MOC (UM)	Heat treated MOC (HM)	Aqueous treated MOC (AM)	Aqueous treated MOC + soluble protein (AMP)	Enzyme treated MOC (EM)	Enzyme treated MOC + soluble protein (EMP)
Available lysine	1.36	1.30	1.25	1.32	1.34	1.33
% of total lysine	82.40	81.25	80.64	82.00	81.21	81.10
Allyl isothiocyanate	0.45	0.35	0.23	0.22	0.20	0.21
% reduction	-	22.22	48.89	51.11	55.56	53.33
Tannins	1.64	1.24	1.14	1.13	1.11	1.14
% reduction	-	24.39	30.48	31.09	32.31	30.48

convector heater. The treatment of supernatant was the same as for the aqueous treatment.

All MOCs treated by the various methods were analysed for proximate and amino acid composition and the results are shown in Tables 6.1 and 6.2 respectively. The levels of available lysine and anti-nutritional factors were also analysed and results are presented in Table 6.3.

6.2.4. Diet Formulation and Preparation

Seven semi-purified isonitrogenous (40% protein) diets were formulated (Table 6.4) using fish meal and mustard oilcake as dietary protein sources. Because of the comparatively lower growth performances of carp fed diets with higher plant protein inclusion levels in previous experiments (Experiments 1 and 2), MOC in this study was tested only at a level of 25% of the total dietary protein.

The Control diet was prepared with fish meal as the sole source of protein. Test diets were formulated using untreated MOC and MOC detoxified by various treatments. Two diets (Diets 5 and 7) were formulated using aqueous and enzyme treated MOC plus coagulated protein from their respective supernatants.

All diets were formulated to contain 0.5% chromic oxide to study the nutrient digestibility of the experimental diets. Carboxymethyl cellulose (CMC) and dextrin were used as binder and carbohydrate source respectively. The composition of mineral and vitamin premixes used are shown in Section 2.2.4. Details of diet formulation and preparation are

TABLE 6.4

Formulation of the experimental diets used in Experiment 4.1

Ingredients	DIETS						
	1 (Control)	2 (UM)	3 (HM)	4 (AM)	5(AMP)	6 (EM)	7EMP)
Untreated MOC		27.05					
Heat treated MOC			27.24				
Aqueous treated MOC				28.69			
Aqueous treated MOC + soluble protein					27.82		
Enzyme treated MOC						28.72	
Enzyme treated MOC + soluble protein							27.84
Fish meal	51.98	38.99	38.99	38.99	38.99	38.99	38.99
Cod liver oil	-	1.03	1.03	1.03	1.03	1.03	1.03
Soybean oil	4.70	2.86	2.92	2.81	2.89	2.80	2.89
Mineral premix[1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin premix[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Binder (CMC)[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
α-cellulose	7.82	5.57	5.32	5.00	5.27	5.00	5.25
Dextrin	27.00	16.00	16.00	14.98	15.50	14.96	15.50

[1] For composition of the mineral and vitamin premixes see Table 2.3 and 2.4 respectively

[2] Sodium carboxymethyl cellulose (high viscosity)

TABLE 6.5

Analysed proximate composition of the experimental diets used in Experiment 4.1

(% dry matter basis unless otherwise stated)

Components	DIETS						
	1 (Control)	2 (UM)	3 (HM)	4 (AM)	5(AMP)	6 (EM)	7(EMP)
Dry matter	94.20	94.54	95.21	94.68	95.45	95.44	94.76
Crude protein	41.25	40.56	41.08	40.37	41.12	40.29	39.85
Crude lipid	9.55	9.60	9.44	10.12	9.76	9.84	9.91
Ash	10.22	11.55	11.76	11.92	11.58	11.90	11.63
Crude fibre	7.96	8.25	8.32	7.98	8.23	8.10	8.24
NFE[1]	31.02	30.04	29.40	29.61	29.31	29.87	30.37
Chromic oxide	0.46	0.48	0.46	0.48	0.48	0.49	0.50
Total energy (Kcal/g)	4.58	4.43	4.46	4.47	4.44	4.46	4.42
PE ratio[2]	90.06	91.55	92.10	90.31	92.61	90.33	90.15
Allyl isothiocyanate[3]	-	0.12	0.10	0.07	0.06	0.06	0.06
Tannins[3]	-	0.44	0.34	0.33	0.31	0.32	0.32

[1] Nitrogen free extractives calculated as
100- (Moisture+Crude protein+Crude lipid+Ash+Crude fibre)

[2] Protein to energy ratio in mg protein/Kcal of total energy

[3] Levels of anti-nutritional factors were estimated from their levels in dietary protein sources determined by various methods (described in Section 2.7.1)

TABLE 6.6

Amino acid composition of the experimental diets used in Experiment 4.1

(% dry matter basis)

Amino acids	DIETS							Requirement for carp[1]
	(Control)	2 (UM)	3 (HM)	4 (AM)	5(AMP)	6 (EM)	7(EMP)	
Arginine	2.62	2.70	2.84	2.72	2.79	2.68	2.70	1.52
Histidine	0.84	1.01	1.05	1.03	1.07	1.00	1.02	0.56
Isoleucine	1.69	1.63	1.70	1.67	1.75	1.64	1.62	0.92
Leucine	3.02	2.94	2.98	2.95	3.01	2.92	2.94	1.64
Lysine	2.86	2.76	2.80	2.74	2.81	2.71	2.74	2.12
Methionine	1.17	1.02	0.99	0.96	0.96	0.98	0.97	0.64
Cystine	0.43	0.47	0.48	0.46	0.46	0.46	0.47	-
Phenylalanine	1.56	1.64	1.66	1.65	1.66	1.62	1.64	1.16
Tyrosine	1.18	1.09	1.10	1.02	1.03	1.03	1.04	-
Threonine	1.66	1.69	1.70	1.70	1.74	1.68	1.69	1.32
Valine	2.06	1.98	2.04	2.02	2.10	2.00	1.98	1.16
Alanine	2.45	2.45	2.46	2.45	2.46	2.43	2.44	-
Aspartic acid	3.94	3.72	3.75	3.70	3.74	3.65	3.66	-
Glutamic acid	5.36	5.89	5.88	5.86	5.89	5.88	5.84	-
Glycine	2.33	2.17	2.15	2.13	2.14	2.12	2.11	-
Proline	1.81	1.93	1.95	1.90	1.87	1.90	1.92	-
Serine	1.40	1.49	1.52	1.50	1.51	1.48	1.47	-

[1] Data for EAA requirement of carp from Ogino (1980)

TABLE 6.7

A summary of the methodology used to study the nutritive value of detoxified MOC on the growth and food utilization in carp

Fish	C. carpio average initial weight 4.91 ± 0.05g
Duration of Experiment	8 weeks
Treatments	Evaluation of detoxified mustard oilcake as dietary protein substitute for fish meal in carp diet
No. of Treatments	7
Replication	3/treatment
Water Temperature	27° ± 1°C
Stocking Density	12/tank
Water Flow Rate	1 litre/min/tank
Feeding Rate	3 times daily (6% of the body weight)
Carcass Sampling	Initial sample - 10 fish at the start of the experiment Final sample - 12 fish per treatment (4 from each replicate)
Faeces Collection	Collected twice daily for two weeks, dried at 60°C in an oven and used for subsequent chemical analysis for digestibility study
Physicochemical characteristics of water monitored	Temperature, pH, dissolved oxygen and total ammonia

described in Sections 2.2.4 and 2.2.5 respectively.

All diets were analysed for proximate and amino acid composition the results of which are presented in Tables 6.5 and 6.6 respectively. The anti-nutritional factors in the diets were calculated using the respective analysed values for the individual dietary plant proteins.

6.2.5. General Experimental Procedure

There were three replicates for each treatment with 12 fish in each replicate. The acclimation and weighing procedures were as described earlier (Section 2.2.3). During the acclimation period fish were fed trout pellet (Ewos Baker's Omega No.3, protein content 49%). Fish were weighed weekly, after 12 hours starvation, during the experimental period. The experiment was conducted for eight weeks. A summary of the methodology used in this experiment is given in Table 6.7.

6.2.6. Feeding Rates

The fish were fed three times daily between 09.00 hours and 17.00 hours at four hourly intervals. Fish were fed at 6% of their body weight per day. Details of food administration are given in Section 3.2.5. The quantity of food delivered per day was adjusted after each weekly weighing and fed for the subsequent week.

6.2.7. Faeces Collection

Fish were transferred to the faecal collection system (Section 2.1.2) at

the beginning of the seventh week and faeces were collected twice daily - morning and evening - for two weeks. Details of the faecal collection procedure are described in Section 3.2.6. Collected faeces were dried in an oven at 60°C. The faecal samples of each replicate tank were pooled to represent respective treatment and kept in air-tight bottles for subsequent chemical analysis.

6.2.8. Histological Techniques

Histoloical techniques were as described in Section 3.2.7. Twelve fish per treatment were sampled for histopathology to assess changes in gills, thyroid, liver, muscle, kidney and intestine.

6.2.9. Analysis of the Experimental Data

Experimental results were analysed as described in Section 2.8.1. Statistical analyses were performed as described in Section 2.9.1.

6.3. RESULTS

6.3.1. Effect of Detoxification on the Chemical Composition of MOC

Proximate and amino acid composition of the detoxified MOCs are presented in Tables 6.1 and 6.2 respectively. The various treatments caused no major changes in proximate and amino acid composition. However, aqueous and enzyme treatments resulted in slight reductions in protein and amino acid contents of the detoxified MOC. The available lysine content of

MOCs did not vary with the treatments (Table 6.3).

There were significant reductions in the allyl isothiocyanate and tannin contents between treatments. The reduction of allyl isothiocyanate content varied between 22.22% and 55.56% with enzyme treatment producing the highest and heat treatment the lowest reduction. The reduction in tannin content varied between 24.39% and 32.31%.

6.3.2. Growth

The growth responses of carp fed the various experimental diets are presented as initial and final mean weights, weight gain, percentage weight gain and specific growth rate (SGR) in Table 6.8 and graphically in Figure 6.1. It appears that inclusion of detoxified MOC in the diet significantly improved growth performances compared to the diet containing untreated MOC. The fish meal based Control diet produced the best growth response throughout the experimental period, while Diet 2 (25% untreated MOC) resulted in poorest growth (Figure 6.1). Diets with both aqueous and enzyme treated MOC produced significantly ($p < 0.05$) better growth performances than the diets with untreated and heat treated MOC.

SGRs for the different treatment groups ranged from 2.8 to 3.09. The SGR for the Control was significantly higher ($p < 0.05$) than for the rest of the diets. There was no significant ($p > 0.05$) difference between SGRs of Diets 2 (untreated MOC) and 3 (heat treated MOC). However, the SGRs of diets 4 (AM), 5 (AMP), 6 (EM), 7 (EMP) were higher than Diets 2 (UM)

TABLE 6.8

Growth and food utilization of common carp fed the experimental diets for eight weeks

Components	DIETS							± S.E.[2]
	1 (Control)	2 (UM)	3 (HM)	4 (AM)	5(AMP)	6 (EM)	7(EMP)	
Initial weight (g)	4.88[a1]	4.88[a]	4.96[a]	4.84[a]	4.93[a]	5.00[a]	4.90[a]	0.05
Final weight (g)	27.66[a]	23.34[f]	23.85[e]	25.12[c]	25.68[b]	25.46[b]	25.08[d]	0.09
Weight gain (g)	22.78[a]	18.46[f]	18.89[e]	20.28[cd]	20.75[b]	20.46[c]	20.18[d]	0.06
% weight gain	467[a]	378[c]	380[c]	419[b]	421[b]	409[b]	412[b]	3.97
SGR (% day)	3.09[a]	2.80[d]	2.80[d]	2.94[bc]	2.95[b]	2.90[c]	2.92[bc]	0.01
SGR as % of Control	100	90.29	90.61	95.14	95.46	93.85	94.17	-
FCR	1.54[b]	1.60[a]	1.58[a]	1.50[c]	1.56[ab]	1.56[ab]	1.50[c]	0.01
PER	1.57[b]	1.54[b]	1.54[b]	1.65[a]	1.56[b]	1.59[b]	1.67[a]	0.02
ANPU (%)	23.27[c]	22.45[f]	22.64[e]	24.32[b]	23.13[d]	23.96[c]	25.02[a]	0.06

[1] Figures in the same row having the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

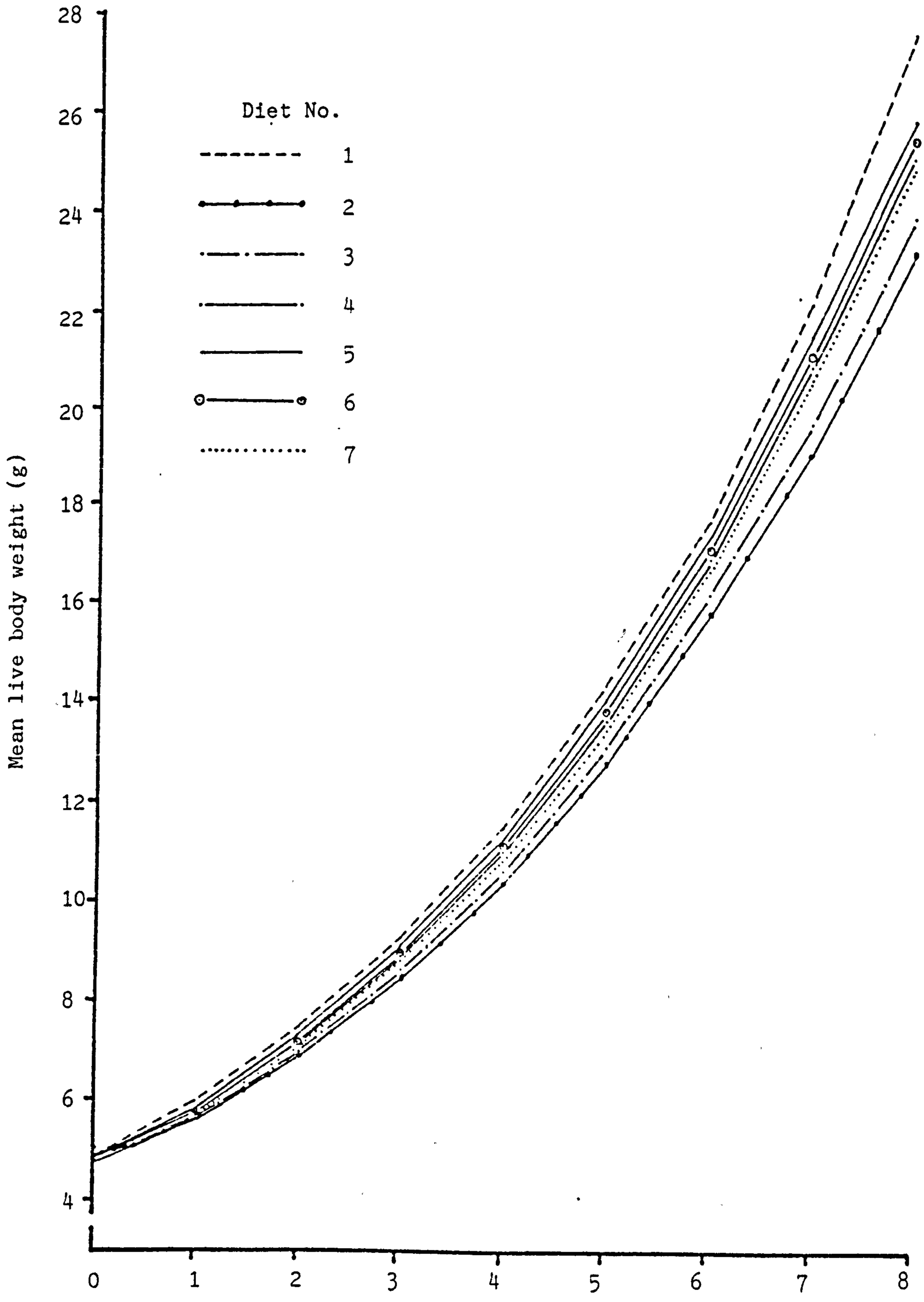


FIGURE 6.1 Growth responses of fish fed experimental diets for 8 weeks

and 3 (HM) (Table 6.8).

6.3.3. Food Conversion Ratio (FCR)

Mean food conversion ratios (FCRs) were calculated for each experimental diet and are presented in Table 6.8. Diets 4 (AM) and 7 (EMP) produced significantly ($p < 0.05$) the lowest FCRs. There were no significant ($p > 0.05$) differences between FCRs of Diets 1 (C), 5 (AMP), 6 (EM) but the FCR of Diet 1 (C) was significantly ($p < 0.05$) lower than that of Diets 2 (UM) and 3 (HM). However, there were no significant differences between the FCRs of Diets 2 (UM), 3 (HM), 5 (AMP) and 6 (EM).

6.3.4. Protein Utilization

Protein utilization efficiency was measured in terms of protein efficiency ratio (PER) and apparent net protein utilization (ANPU %) (Table 6.8). PERs followed similar trends to FCRs with Diets 4 (AM) and 7 (EMP) producing significantly ($p < 0.05$) the highest PERs. However, there were no significant ($p > 0.05$) differences between PERs of the Control and Diets 2 (UM), 3 (HM), 5 (AMP) and 6 (EM).

Mean apparent net protein utilization (ANPU %) was calculated for all dietary treatments and ranged from 22.45% to 25.02% (Table 6.8). Diet 7 (EMP) produced significantly ($p < 0.05$) the highest and Diet 2 (UM) the lowest ANPU. However, there was no significant ($p > 0.05$) difference between ANPUs of Diets 1 and 6, but these values were significantly ($p < 0.05$) higher than for the rest of the diets.

6.3.5. Apparent Nutrient Digestibility

Apparent nutrient digestibilities of the experimental diets were determined as described earlier (Section 2.8.1) and the results are presented in Table 6.9. Apparent dry matter digestibility values for different dietary treatments ranged from 67.12% to 78.5% with the Control diet having the highest value followed by Diet 6 (EM). All the diets showed fairly high apparent protein digestibility ranging from 83.42% to 88.85% with the Control having the highest and Diet 7 (EMP) the lowest. The apparent lipid, ash and energy digestibilities ranged from 87.92% to 93.33%, 28.72% to 44.07% and 75.02% to 83.13% respectively. Again, the Control diet had the highest lipid, ash and energy digestibility.

6.3.6. Carcass Composition

The results of proximate carcass composition of the fish at the start and at the end of the experiment are presented in Table 6.10. Fish from Diet 2 (UM) had significantly ($p < 0.05$) the highest moisture (78.58%) and lowest lipid content (4.36%). Fish fed the Control diet had significantly ($p < 0.05$) the lowest moisture and highest lipid content. However, there were no significant differences ($p > 0.05$) between carcass moisture contents of fish fed Diets 2 (UM), 3 (HM), 4 (AM), 5 (AMP), 6 (EM) and 7 (EMP).

There were no significant ($p > 0.05$) differences between the carcass protein contents of fish fed Diets 1 (Control), 4 (AM), 6 (EM) and 7

TABLE 6.9

Apparent nutrient digestibility (%) of the experimental diets

	DIETS						
	1 (Control)	2 (UM)	3 (HM)	4 (AM)	5(AMP)	6 (EM)	7(EMP)
Apparent dry matter digestibility	78.5	69.03	68.49	67.12	67.56	69.37	67.74
Apparent protein digestibility	88.85	83.85	84.18	84.61	84.09	84.56	83.42
Apparent lipid digestibility	93.33	88.09	90.35	89.21	90.76	87.92	89.19
Apparent ash digestibility	44.07	36.44	36.50	36.61	33.76	34.81	28.72
Apparent energy digestibility	83.19	74.27	74.64	74.92	75.02	76.31	75.18

* No statistical analysis was possible as determinations were performed on pooled samples

TABLE 6.10

Proximate carcass composition (% fresh matter basis) of fish samples at the start and end of the experiment

Components	Initial (all fish)	FINAL DIET NO. 1 (Control)	2 (UM)	3 (HM)	4 (AM)	5(AMP)	6 (EM)	7(EMP)	± S.E.[2]
Moisture	80.71	77.44^{b}	78.58^{a1}	78.42^{a}	78.37^{a}	78.27^{a}	78.17^{a}	78.26^{a}	0.14
Crude protein	13.26	14.81^{a}	14.30^{d}	14.38^{cd}	14.60^{abc}	14.43^{bcd}	14.69^{ab}	14.54^{abcd}	0.08
Crude lipid	3.32	5.14^{a}	4.36^{b}	4.37^{b}	4.44^{b}	4.58^{b}	4.51^{b}	4.48^{b}	0.08
Ash	2.31	2.30^{c}	2.43^{ab}	2.45^{a}	2.37^{b}	2.40^{ab}	2.40^{ab}	2.40^{ab}	0.02
TOTAL	99.60	99.69	99.67	99.62	99.78	99.68	99.77	99.71	-

[1] Figures in the same row with the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

(EMP) but these values were significantly ($p < 0.05$) higher than recorded for the rest of the diets.

The Control diet produced significantly ($p < 0.05$) the highest carcass lipid content but there were no significant differences ($p > 0.05$) between the carcass lipid contents of fish fed the rest of the diets. Carcass ash contents ranged from 2.30% to 2.40%.

6.3.7. General Health and Histological Examination

At the end of the experiment, no abnormalities or physical deformities were observed in fish examined from the various dietary treatment groups. Histological examination of various organs such as gills, liver, thyroid, kidney, muscle and intestine of fish from various dietary treatments revealed no significant changes in these tissues.

6.4. DISCUSSION

Detoxification of MOC by aqueous extraction and enzyme treatment resulted in only minor changes in the proximate composition, particularly the crude protein content (Table 6.1). Similarly minor changes were found in amino acid composition (Table 6.2). Ballester et al. (1970) reported a similar slight reduction in crude protein content from 37.2% to 35.6% in rapeseed meal detoxified by aqueous extraction. The slight loss of crude protein from MOC detoxified by aqueous extraction and enzyme treatment may be due to loss of water soluble proteins.

Although no significant changes in available lysine content were observed with the various processing treatments both heat and aqueous treatment of MOC resulted in slight reductions in available lysine content (Table 6.4). Ballester et al. (1970) also found similar minor reductions (4.42% to 3.69%) of available lysine in rapeseed meal detoxified by aqueous extraction. Abel et al. (1984) found that the available lysine content in full-fat soybean was not affected by heat treatment. On the other hand, Mustakas et al. (1965) reported a significant reduction (about 22%) in lysine content of rapeseed meal during heat processing, particularly when the meals were dried excessively during cooking or when the defatted and desolventized meals were steam heated. One possible explanation of the reduction of available lysine would be that exposure of lysine to heating may have led to the binding of the ε-amino gorups with other compounds.

Among the various methods employed to detoxify MOC, heat treatment appeared to be the least effective in reducing the allyl isothiocyanate and tannin levels (Table 6.3). However, the reduction of allyl isothiocyanate by heat treatment was found to be about 22% which is comparatively lower than the values found by other workers. Rutkowski (1970) reported that heat treatment at 120°C was able to destroy more than 50% of isothiocyanate in rapeseed meal. Bell et al. (1981) also reported that heat treatment alone resulted in significant reduction (about 50% of the original) in glucosinolates (especially allyl-type) in mustard seed (B. juncea).

Both aqueous and enzyme treatments were effective in reducing the

isothiocyanate content of MOC (Table 6.3). Ballester et al. (1970) reported that double extraction of water (first 12h water extraction followed by second extraction at room temperature) resulted in a reduction of 77% isothiocyanate in rapeseed meal. In another study, Ballester et al. (1973) found that a 2h continuous water extraction with stirring completely removed isothiocyanates. However, the comparatively lower reduction (about 50%) of isothiocyanate by aqueous extraction found in this study could be due to the differences in techniques employed, such as double extraction and continuous extraction with stirring.

In the present study enzymatic treatment was the most effective in reducing isothiocyanate in MOC. This is likely to be due to the fact that incubation of MOC with water at 60°C for 1 hour initiated the enzymatic hydrolysis of glucosinolate and steam distillation resulted in removal of volatile isothiocyanate. Goering (1963) and Mustakas et al. (1963) also found the enzymatic method effective in reducing isothiocyanate in rapeseed meal and mustard seed (B. juncea) respectively. Shah et al. (1977) reported that the maximum detoxification of MOC can be accomplished by enzymatic or aqueous treatment.

The tannin content in MOC was less affected by the various processing treatments. However, water extraction of MOC resulted in a reduction of about 31% of tannin. Singh and Arrora (1978) observed a similar reduction (about 35%) of tannins in salseed (Shorea robusta) meal after water extraction at room temperature for 24 hours. Heat treatment of MOC resulted in a reduction of about 24% tannins whereas Price et al. (1978) reported that autoclaving of grain sorghum (Br-54) for 20 minutes (21

132°C) decreased the tannin content by 83%.

The results of the present growth trial demonstrated that the use of detoxified MOC in carp diets improved the growth performance of fish. Excluding the Control, Diet 5 (AMP) produced significantly ($p < 0.05$) the highest weight gain among the experimental diets followed by Diet 6 (EM). This weight gain was 91% of that obtained for fish fed the fish meal based Control diet. Ballester et al. (1973) reported that rapeseed meal detoxified by water extraction gave a PER and growth rate similar to casein diets in rats. Shah et al. (1977) also found improved growth in rats fed aqueous treated mustard oilcake supplemented with coagulated protein from the supernatant. Lower allyl isothiocyanate contents and addition of coagulated protein (soluble protein) from the supernatant to detoxified MOC probably contributed to the better growth performance of fish on Diet 5 (AMP).

Diet 6 (EM) produced weight gains similar to that of Diet 4 (AM) but significantly ($p < 0.05$) higher than that of Diets 2 (UM) and 3 (HM) (Table 6.8). Diet 6 (EM) produced a weight gain about 90% of the Control. Belzile and Bell (1966) observed improvements in the growth performance of rats using enzyme treated rapeseed meal. Shah et al. (1977) also observed improved growth in rats with enzyme treated mustard oilcake and the growth performance was more pronounced when the detoxified MOC was supplemented with the coagulated protein from the supernatant.

The growth performance of fish on Diet 3 (HM) was significantly ($p < 0.05$) higher than Diet 2 (untreated MOC) but lower than for the rest of the diets. Srivastava and Hill (1976) reported that heat treatment resulted in greater weight gain and lower thyroid weight in rats fed diets containing low-glucosinolate rapeseed meal, although the weight gains were less than that obtained with a soybean based Control diet.

Abel et al. (1984) also found that carp fed soybean treated with thermally and hydrothermally produced higher growth performances than for fish fed a commercially available feed. Rats fed autoclaved high tannin sorghum grain appeared to grow at a more rapid rate and exhibit better feed efficiency than those fed the untreated grains (Price et al., 1978).

Although heat treatment prevents further breakdown of the thioglucoside in the meal, the possibility remains that the glucosides which are allowed to remain in the meal could be subsequently hydrolysed to toxic end products, after ingestion, by enzymes produced in the intestinal tract (Marangos and Hill, 1974). This might explain the comparatively lower growth performance of fish fed Diet 3 (HM).

Diet 2 (UM) showed the poorest growth response and food utilization. This observations is similar to the findings of Capper et al. (1982) who found poor growth rate and food utilization of carp fed diets containing 20% untreated mustard oilcake. Similar reductions in growth rate and food utilization have been recorded in trout and salmon fed diets containing more than 30% and 25% protein respectively from rapeseed meal (Yurkowski et al., 1978; Higgs et al., 1982).

The overall food and protein utilization also improved with the use of detoxified MOC. The food conversion ratio was lowest in Diets 3 (1.50) and 7 (1.50) and increased in order Diets 2 (1.54), 5 (1.56), 6 (1.56), 3 (1.58) and 2 (1.60). The FCRs, PERs and ANPUs obtained in this experiment are better than those in Experiment 1 (Table 3.9) using the same percentage inclusion of MOC protein.

The FCRs obtained in this trial are also comparatively better than those reported for carp (2.86) fed soybean diets by Atack et al. (1979) and for tilapia (1.86) fed diets containing 25% rapeseed meal (Jackson et al., 1982).

Use of detoxified MOC improved the ANPU in experimental diets (Table 6.8). Ballester et al. (1970) also reported that simple water extraction of rapeseed meal improved the NPU values from 40% to 62% in rats.

Apart from the fish meal based Control diet all the test diets showed similar dry matter digestibilities (Table 6.9) which are comparatively higher than those found in Experiment 1 (Table 3.10). Ballester et al. (1970) found that simple water extraction of rapeseed meal improved the protein digestibility from 67% to 71%. However, in this present study detoxification of MOC did not improve the apparent protein digestibility (Table 6.9). Srivastava and Hill (1976) also reported that heat treatment did not improve the digestibility of rapeseed meal in rats.

The fish meal based Control diet showed the highest (83.19%) energy digestibility among the experimental diets (Table 6.9). This is lower

than the reported energy digestibility value of fish meal for trout (91.5%, Smith et al., 1980) and higher than reported for trout (74%, Windell et al., 1978b) but similar to that for grass carp (83%, Law, 1986). However, detoxification of MOC slightly improved the energy digestibility of the different experimental diets (Table 6.9).

Fish fed the Control diet showed significantly ($p < 0.05$) the lowest carcass moisture but highest protein and lipid content. However, there were no significant variations among the carcass moisture contents of fish fed the other diets.

There was an inverse relationship between lipid and moisture content in accordance with the findings of other workers (Dabrowska and Wojno, 1977; Zeitler, 1984). The reduced carcass lipid in fish fed diets containing MOC in this study is well supported by the findings of Dabrowski and Kozlowska (1981) in carp; Yurkowski et al. (1978) in rainbow trout, and Higgs et al. (1979) in coho salmon fed diets containing rapeseed meal.

The results of the present study suggest that both aqueous and enzymatic treatments were effective in reducing the anti-nutritional factors in mustard oilcake. The use of detoxified mustard oilcake in diets improved the growth performance and food utilization in carp compared to untreated mustard oilcake.

EXPERIMENT 4.2

Detoxification of linseed and sesame meal and evaluation of their nutritive value in the diet of common carp (*C. carpio* L)

6.5 INTRODUCTION

It is well known that a variety of plant proteins are potentially toxic because they contain glucosides which release HCN upon hydrolysis (Montgomery, 1964). Those plants which contain high levels of such cyanogenetic glucosides include cassava, lima beans, sorghum and linseed. The glucoside itself is not toxic, but the HCN which can be released upon hydrolysis by an endogenous enzyme is. The toxic principle in linseed is linamarin or phaseolunatin from which acetone and HCN are released by a heat labile autoenzyme, linamarase (Montgomery, 1964). However, reductions in toxicity of such feedstuffs may be accomplished by processing (e.g. water extraction, boiling, roasting or sun-drying) which serves to inactivate the enzyme and volatilize any HCN that may have been released.

Several investigators have shown that linseed meal gave poor growth when substituted for protein supplements in chick starter diets. McGinnis and Polis (1946) evaluated several methods of treating linseed meal to improve its nutritive value for chicks. They found that a marked improvement resulted when the linseed meal was water-treated. This treatment consisted of wetting of the linseed meal with approximately twice its weight of water and allowing the mixture to stand for 18 hours at 37°C. The linseed meal treated in this manner gave a greatly increased growth response when compared with untreated meal as the only source of supplementary protein. Kratzer (1946, 1947) showed that "water treatment" in which a 1:3 ratio of linseed and tap water mixture was allowed to stand at 22°C for 12 hours (with subsequent drying before

incorporation into the diet) gave as good a growth response as longer treatments.

McGregor and McGinnis (1948), in similar studies, reported that linseed meal contains a substance which depressed chick-growth when linseed meal was added to an otherwise adequate diet. Water extracted linseed meal (2.5 water:1 linseed meal at 25°C for 18 hours) at 30% of the diet did not cause growth depression in chicks. These authors concluded that the toxic factor in linseed meal was apparently destroyed by simple water treatment.

Kratzer and Williams (1948) suggested that linseed meal was toxic because its ingestion produced a deficiency in one or more B-complex vitamins. They proposed a deficiency caused by the formation of a complex with these vitamins rendering them unavailable. In another study with turkey poults Kratzer (1949) observed improved growth and survival rates with water extracted or pyridoxine supplemented meal but not with pyridoxine supplementation of water extracted meal. Kratzer et al. (1954) reported that linseed meal depressed growth of chicks when fed at a level of 30% of the diet. Water extraction and autoclaving were effective in destroying the growth depressing factors while dry-heat was ineffective.

Mani et al. (1949) reported an interesting study which suggested that the mucilage of flaxseed (linseed) hull might affect digestibility because of the viscous nature of the wetted material. Addition of flaxseed mucilage to normal rations produced a retardation in growth similar to the effect of untreated linseed meal, including low food intake and high

death rate. The authors subjected mucilage to prolonged water treatment, dry-heating and autoclaving at conditions similar to those which improved the nutritive value of linseed and showed each of these treatments were effective in decreasing the viscosity of the mucilage solution.

Singh and Punia (1979) detoxified linseed meal by extracting in ordinary and acidified water (1% HCl) with subsequent autoclaving at 1 Kg/cm^2 for 15 minutes. The use of water extracted autoclaved meal at 50% replacement of groundnut meal in poultry rations produced growth comparable to groundnut meal whilst the acidified water extracted meal was slightly inferior.

Phytate (a cyclic compound, inositol) contains six phosphate radicals capable of binding with other metal ions such as Ca, Mg, Zn and Fe to form poorly soluble compounds which are not readily absorbed from the intestine (Oberleas, 1973). Both linseed and sesame meals contain phytic acid. However, the ability of phytic acid to bind metal ions is lost when the phosphate groups are hydrolysed through the action of the enzyme phytase (Liener, 1977).

A 50% decrease in phytate content was observed when whole soybeans were soaked in water for 24 hours at room temperature (Anon, 1976; cited by Cheryan, 1980). However, only a trace of phytate was found in the soak water. At 55°C about 90% of the phytic acid was removed and significant amounts were found in the soak water. These results suggest a breakdown of phytic acid during soaking, although these workers were not able to demonstrate the presence of phytase in soybean.

Joseph (1973) observed a decrease in phytate in cassava by soaking, also probably due to partial hydrolysis.

Autoclaving reduced the chick requirement for supplementary zinc when fed isolated soybean protein or sesame diets (Kratzer et al., 1959; Lease et al., 1960). It has been suggested that this reduction was due to the destruction of phytic acid (O'Dell et al., 1964). O'Dell (1962) found that four hour autoclaving reduced the phytic acid content of isolated soybean protein from 2.68% to 0.36%. Lease (1966), on the other hand, reported that autoclaving of sesame meal for four hours only reduced the phytic acid content from 1.00% to 0.78%, an insignificant difference. However, inclusion of autoclaved sesame meal led to a significant increase in growth, no leg deformities and an increase in the zinc content of the bone.

From the foregoing discussion, it is seen that the toxic factors in linseed and sesame meal are reported to be heat labile and/or water soluble. Hence, an attempt was made to detoxify both linseed and sesame meals by water extraction and heat treatment and to evaluate their nutritive value in the diets of common carp.

6.6 MATERIALS AND METHODS

6.6.1. Experimental System and Animals

The experimental systems used in this experiment are described earlier (Section 2.1.1 and 2.1.2). The sources of experimental animals and their quarantine procedures are described in Section 2.2.1.

6.6.2. Analytical Techniques

The analytical techniques used were as described in Chapter 2.

6.6.3. Detoxification of Linseed and Sesame Meal

Both linseed (Linum usitatissimum) and sesame meal (Sesamum indicum) were of expeller type and obtained from Bangladesh. Detoxification of linseed and sesame meals was accomplished by heat treatment and aqueous treatment as follows:

(i) Heat treatment

The ground linseed and sesame meals were subjected to steam autoclaving for two hours at 1 Kg/cm^2 pressure in an autoclave. The meals were then dried at 40°C using an electric fan convector heater.

(ii) Aqueous treatment

Aqueous extraction of linseed and sesame meal was carried out according to Ballester et al. (1970). The detailed procedure was as described for mustard oilcake in Section 6.2.3.

All the detoxified meals were analysed for proximate and amino acid composition and the results are shown in Tables 6.11 and 6.12 respectively. The available lysine and the anti-nutritional factors were also analysed and results are presented in Table 6.13.

6.6.4. Diet Formulation and Preparation

Seven semi-purified, isonitrogenous (40%), diets were formulated (Table

TABLE 6.11

Proximate composition of dietary ingredients used in Experiment 4.2

(% dry matter basis)

	INGREDIENTS						
	Fish meal	Untreated linseed (UL)	Heat treated linseed (HL)	Aqueous treated linseed (AL)	Untreated sesame meal (US)	Heat treated sesame (HS)	Aqueous treated sesame (AS)
Dry matter	92.23	89.65	89.00	92.26	87.60	88.10	94.50
Crude protein	76.95	32.96	32.95	33.35	36.69	36.73	36.91
Crude lipid	10.19	2.81	2.80	2.52	2.48	2.46	2.20
Ash	11.73	11.86	11.80	11.90	13.20	13.21	12.94
Crude fibre	0.55	9.08	9.12	9.14	23.11	23.00	23.20
NFE[1]	0.57	43.29	43.33	43.09	24.52	24.60	24.75

[1] Nitrogen free extractives calculated as

100 - (Moisture+Crude protein+Crude lipid+Ash+Crude fibre)

TABLE 6.12

Amino acid composition (% dry matter) of the dietary protein sources used in Experiment 4.2

	PROTEIN SOURCES						
	Fish meal	Untreated linseed (UL)	Heat treated linseed (HL)	Aqueous treated linseed (AL)	Untreated sesame (US)	Heat treated sesame (HS)	Aqueous treated sesame (AS)
Arginine	5.06	2.18	2.20	2.18	4.15	4.20	4.23
Histidine	1.60	0.68	0.68	0.70	0.80	0.80	0.78
Isoleucine	3.30	1.31	1.30	1.32	1.19	1.20	1.22
Leucine	5.71	1.84	1.83	1.84	1.92	1.94	1.95
Lysine	5.46	1.28	1.26	1.26	1.24	1.20	1.23
Methionine	2.22	0.26	0.26	0.27	0.28	0.27	0.28
Cystine	0.85	0.38	0.37	0.38	0.56	0.56	0.58
Phenylalanine	2.82	1.52	1.50	1.54	1.55	1.57	1.56
Tyrosine	2.10	0.66	0.65	0.66	0.78	0.76	0.77
Threonine	3.19	1.25	1.26	1.25	1.24	1.23	1.24
Valine	4.02	1.70	1.68	1.72	1.63	1.60	1.62
Alanine	4.65	1.62	1.60	1.61	1.63	1.62	1.64
Aspartic acid	7.60	3.16	3.10	3.14	3.18	3.14	3.20
Glutamic acid	10.35	6.15	6.17	6.17	6.17	6.18	6.21
Glycine	4.46	2.10	2.11	2.12	1.65	1.61	1.64
Proline	3.51	1.34	1.32	1.34	1.60	1.58	1.59
Serine	2.62	1.43	1.41	1.44	1.58	1.55	1.60

TABLE 6.13

Effect of different processing treatments on available lysine, phytic acid and hydrocyanic acid contents (% dry matter basis) of linseed and sesame meals

	Untreated linseed meal (UL)	Heat treated linseed meal (HL)	Aqueous treated linseed meal (AL)	Untreated sesame meal (US)	Heat sesame meal (HS)	Aqueous treated sesame meal (AS)
Available lysine	1.06	1.03	1.04	1.00	0.96	0.99
% of total lysine	82.81	81.74	82.54	80.64	80.00	80.48
Phytic acid	2.45	0.69	1.27	2.38	0.62	1.17
% reduction	-	71.84	48.16	-	73.95	50.84
Hydrocyanic acid	0.032	0.021	0.015	-	-	-
% reduction	-	34.38	53.12	-	-	-

6.14) using fish meal, linseed and sesame meals as dietary protein sources. As for mustard oilcake, linseed and sesame meals were tested only at a level of 25% of total dietary protein. The Control diet was prepared with fish meal as the sole source of protein. Test diets were formulated using untreated, heat treated and aqueous treated linseed and sesame meals (Table 6.14). All the diets were formulated to contain 0.5% chromic oxide to study nutrient digestibility.

The composition of the vitamin and mineral premixes used are shown in Section 2.2.4. Carboxymethyl cellulose and dextrin were used as binder and carbohydrate source respectively. Details of diet formulation and preparation are described in Sections 2.2.4 and 2.2.5 respectively. All the diets were analysed for proximate and amino acid composition and results are presented in Tables 6.15 and 6.16 respectively. Hydrocyanic acid in the diets was calculated from its level in linseed meal determined by the method described in Section 2.7.1.

6.6.5. General Experimental Procedure

Each treatment had three replicates, 12 fish per replicate, with a mean initial weight of 3.3g±0.04g. Acclimation and weighing procedures were as described earlier (Section 2.2.3). During the acclimation period fish were fed trout pellet (Ewos Baker's Omega No.3, 49% protein). Weighing of the fish during the experiment was carried out every seven days, after 12 hours of starvation. The experiment was conducted for a period of eight weeks. A summary of the methodology used in the present investigation is given in Table 6.17.

TABLE 6.14

Composition of the experimental diets used in Experiment 4.2

	DIET NO						
	1 (Control)	2 (UL)	3 (HL)	4 (AL)	5 (US)	6 (HS)	7 (AS)
Untreated linseed meal		30.34					
Heat treated linseed meal			30.35				
Aqueous treated linseed meal				29.99			
Untreated sesame meal					27.26		
Heat treated sesame meal						27.23	
Aqueous treated sesame meal							27.09
Fish meal	51.98	38.99	38.99	38.99	38.99	38.99	38.99
Cod liver oil	-	1.03	1.03	1.03	1.03	1.03	1.03
Soybean oil	4.70	4.15	4.15	4.25	4.33	4.33	4.40
Mineral mixture[1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin premix[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Binder (CMC)[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
α-cellulose	8.00	5.00	5.00	5.00	2.00	2.00	2.00
Dextrin	26.82	12.00	11.98	12.24	17.89	17.92	

[1] For composition of mineral and vitamin premix see Tables 2.3 and 2.4 respectively

[2] Sodium carboxymethyl cellulose (high viscosity)

TABLE 6.15

Analysed proximate composition of the experimental diets used in Experiment 4.2
(% dry matter basis unless otherwise stated)

Components	DIET NO.						
	1 (Control)	2 (UL)	3 (HL)	4 (AL)	5 (US)	6 (HS)	7 (AS)
Dry matter	94.59	94.33	94.02	94.80	93.96	94.56	95.14
Crude protein	39.66	39.87	40.50	40.41	39.83	40.09	41.40
Crude lipid	10.20	9.85	10.27	10.08	9.87	10.18	9.93
Ash	10.08	11.92	11.97	11.86	12.10	12.12	11.90
Crude fibre	7.88	7.52	7.35	7.96	8.19	8.38	8.30
NFE[1]	32.18	30.84	29.91	29.69	30.01	29.23	28.47
Chromic oxide	0.49	0.52	0.50	0.50	0.51	0.49	0.49
Total energy (Kcal/g)	4.52	4.43	4.47	4.48	4.43	4.42	4.44
PE Ratio[2]	87.74	90.00	90.60	90.20	89.91	90.70	93.24
Phytic acid	-	0.73	0.20	0.37	0.66	0.16	0.31
Hydrocyanic acid[3]	-	0.01	0.006	0.004	-	-	-

[1] Nitrogen free extractives calculated as
100 - (Moisture+Crude protein+Crude lipid+Ash+Crude fibre)

[2] Protein to energy ratio in mg protein/Kcal of total energy

[3] Hydrocyanic acid was estimated from its level in dietary protein source determined by the method described in Section 2.7.1

TABLE 6.16

Amino acid composition of the experimental diets used in Experiment 4.2

(% dry matter basis)

Amino acids	DIET NO.							Requirement for carp[1]
	1 (Control)	2 (UL)	3 (HL)	4 (AL)	5 (US)	6 (HS)	7 (AS)	
Arginine	2.64	2.63	2.57	2.66	3.12	3.12	3.20	1.52
Histidine	0.85	0.83	0.80	0.86	0.83	0.84	0.86	0.56
Isoleucine	1.72	1.69	1.70	1.74	1.64	1.62	1.69	0.92
Leucine	3.10	2.79	2.86	2.75	2.72	2.75	2.80	1.64
Lysine	2.91	2.52	2.61	2.48	2.46	2.51	2.54	2.12
Methionine	1.19	0.93	0.92	0.96	0.94	0.92	0.96	0.64
Cystine	0.44	0.45	0.45	0.43	0.47	0.44	0.49	-
Phenylalanine	1.54	1.56	1.51	1.54	1.51	1.49	1.55	1.16
Tyrosine	1.15	1.02	1.07	1.00	1.01	1.00	1.08	-
Threonine	1.68	1.62	1.63	1.60	1.58	1.65	1.73	1.32
Valine	2.10	2.09	2.14	2.07	2.02	2.00	2.05	1.16
Alanine	2.41	2.30	2.31	2.35	2.26	2.28	2.33	-
Aspartic acid	3.90	3.92	3.95	3.96	3.78	3.80	3.91	-
Glutamic acid	5.41	5.89	5.81	5.92	5.72	5.70	5.84	-
Glycine	2.36	2.38	2.41	2.40	2.22	2.24	2.30	-
Proline	1.78	1.78	1.80	1.76	1.83	1.80	1.86	-
Serine	1.44	1.45	1.48	1.44	1.47	1.50	1.56	-

[1]Data for essential amino acid requirement of carp from Ogino (1980)

TABLE 6.17

A summary of the methodology used to study the nutritive value of detoxified linseed and sesame meals on the growth and food utilization in carp

Fish	C. carpio, mean initial weight 3.3 ± 0.04g
Duration of Experiment	Eight weeks
Treatments	Evaluation of detoxified linseed and sesame meal as dietary protein substitute for fish meal in carp diet
No. of Treatment	Seven
Replication	3/treatment
Water Temperature	27° ± 1°C
Stocking Density	12/tank
Water Flow Rate	1 litre/min/tank
Carcass Sampling	Initial sample - 10 fish at the start of the experiment Final sample - 12 fish per treatment (4 fish from each replicate)
Faeces Collection	Collected twice daily for two weeks, dried at 60°C in an oven and used for subsequent chemical analysis for digestibility study
Physico-chemical characteristics of water monitored	Temperature, pH, dissolved oxygen and total ammonia

6.6.6. Feeding Rate

The fish were fed three times daily between 09.00 hours and 17.00 hours at four hourly intervals. Fish in each tank were fed 6% of their total biomass per day. As the moisture contents of all the diets were low and similar, no correction of feeding rate was made for moisture content. Details of food administration are given in Section 3.2.4. A record of amount of food fed was kept for subsequent calculation of food conversion and protein utilization. The quantity of food fed per day was adjusted after each weekly weighing and fed for the subsequent week.

6.6.7. Faeces Collection

Fish were transferred to the faecal collection system (Section 2.1.2) at the beginning of the seventh week and faeces were collected twice daily - morning and evening - for two weeks. Details of faecal collection procedure are described in Section 3.2.5.

6.6.8. Histological Techniques

Histological techniques were as described in Section 3.2.7. Twelve fish per treatment were sampled for histopathology to assess changes in gills, thyroid, liver, muscle, kidney and intestine.

6.6.9. Analysis of Experimental Data

Experimental results were analysed as described in Section 2.8.1. Statistical analyses were performed as described in Section 2.9.1.

6.7 RESULTS

6.7.1. Effect of Detoxification on the Chemical Composition of Linseed and Sesame Meals

Proximate and amino acid composition of the detoxified linseed and sesame meals are presented in Tables 6.11 and 6.12 respectively. The various processing treatments caused no major changes in proximate or amino acid composition. Aqueous treatment had no effect on the available lysine content (Table 6.13). However, heat treatment resulted in slight reduction in available lysine content in both linseed and sesame meals. There were significant reductions in the phytic acid and hydrocyanic acid contents due to the various treatments. The reduction in phytic acid content varied between 48.16% and 73.95% with heat treatment producing the greatest reduction (Table 6.13). The reduction of hydrocyanic acid in linseed meal varied between 34.38% and 53.12%.

6.7.2. Growth

The growth performance of carp fed the various experimental diets are presented as mean initial and final weights, weight gain, percentage weight gain and specific growth rate (SGR) in Table 6.18 and Figure 6.2. Inclusion of detoxified linseed and sesame meals in the diets significantly ($p < 0.05$) improved growth performances compared to the diets containing untreated linseed and sesame meals. The fish meal based Control diet produced the best growth responses throughout the experimental period whilst Diet 2 (UL) produced poorest growth (Figure 6.2).

TABLE 6.18

Growth and food utilization of common carp fed the experimental diets for eight weeks

Components	DIET NO.							± S.E.[2]
	1 (Control)	2 (UL)	3 (HL)	4 (AL)	5 (US)	6 (HS)	7 (AS)	
Mean initial weight (g)	3.33^{a1}	3.25^{a}	3.28^{a}	3.28^{a}	3.26^{a}	3.34^{a}	3.35^{a}	0.03
Mean final weight (g)	20.12^{a}	15.86^{f}	16.08^{e}	17.60^{c}	16.63^{d}	19.28^{b}	17.41^{c}	0.06
Weight gain (g)	16.79^{a}	12.61^{g}	12.80^{f}	14.32^{c}	13.37^{e}	15.94^{b}	14.06^{d}	0.03
% weight gain	504^{a}	388^{e}	390^{e}	438^{c}	410^{d}	477^{b}	420^{d}	3.35
SGR (% day)	3.21^{a}	2.83^{bc}	2.84^{b}	3.00^{ab}	2.64^{c}	3.13^{a}	2.94^{ab}	0.09
SGR as % of Control	100	88.16	88.47	93.45	82.24	97.50	91.58	-
FCR	1.50^{de}	1.60^{b}	1.65^{a}	1.54^{c}	1.53^{cd}	1.48^{e}	1.56^{c}	0.01
PER	1.69^{a}	1.56^{d}	1.50^{e}	1.60^{c}	1.65^{b}	1.69^{a}	1.55^{d}	0.01
ANPU %	25.58^{a}	22.60^{e}	22.43^{e}	24.12^{b}	23.73^{c}	25.55^{a}	23.10^{d}	0.06

[1] Figures in the same row having the same superscripts are not significantly ($p > 0.05$) different

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

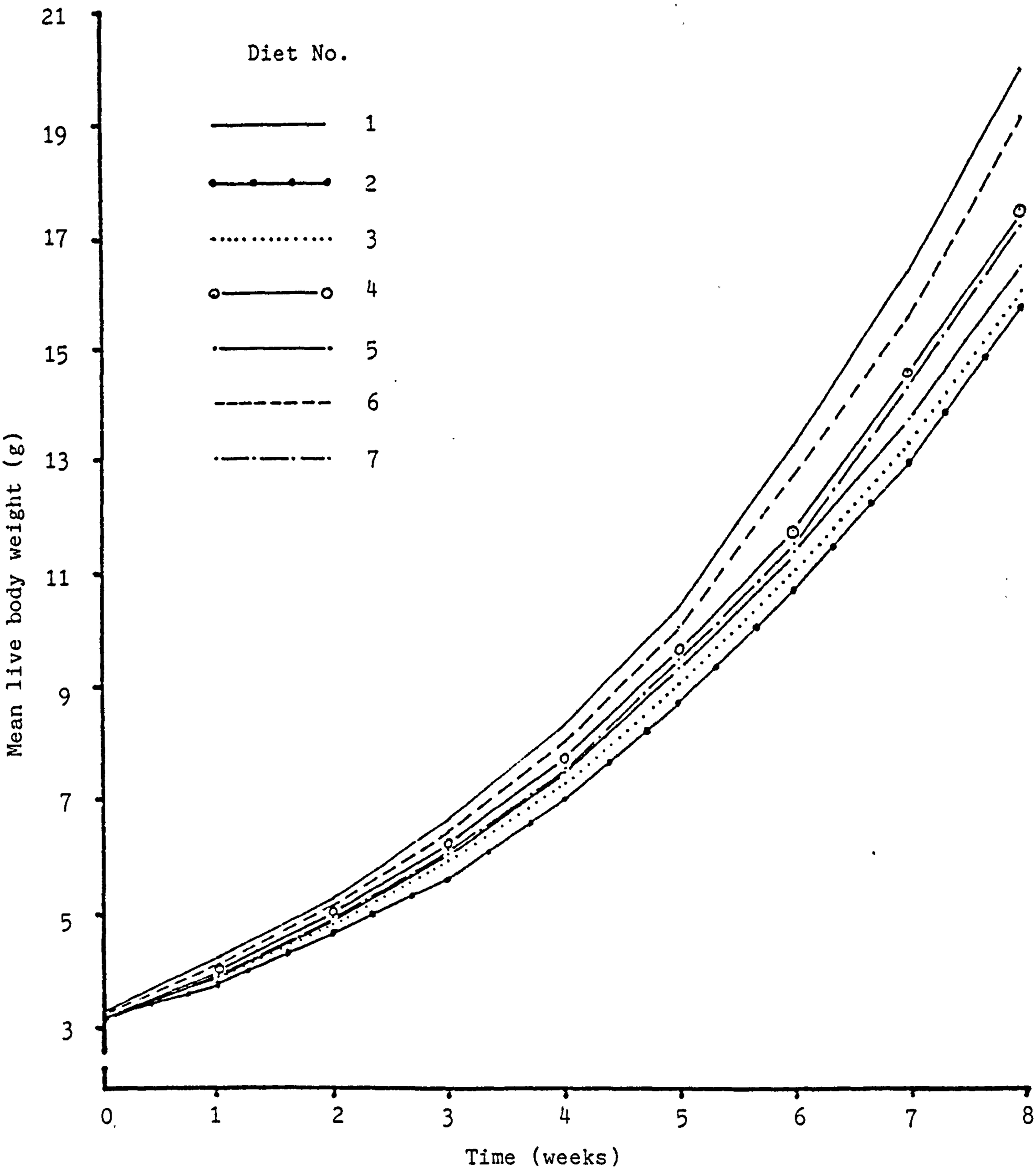


FIGURE 6.2 Growth responses of fish fed experimental diets for 8 weeks

Apart from the Control, Diet 6 (HS) produced significantly ($p < 0.05$) the best growth performance followed by Diet 4 (AL).

SGRs for the different dietary treatment groups ranged from 2.64 to 3.21. There were no significant ($p > 0.05$) differences between the SGRs of the Control and Diets 4 (AL), 6 (HS), and 7 (AS). However, the SGRs of both control and Diets 6 (HS) were significantly ($p < 0.05$) higher than those for the rest of the diets (Table 6.18). Again, there were no significant ($p > 0.05$) differences between the SGRs of the Diets 2 (UL), 3 (HL), 4 (AL), and 7 (AS).

6.7.3. Food Conversion Ratio (FCR)

Mean food conversion ratios were calculated for each experimental diet and are presented in Table 6.18. Both the Control and Diet 6 (HS) produced significantly ($p < 0.05$) the lowest FCRs. However, there was no significant ($p > 0.05$) difference between the FCRs of Diet 1 (Control) and 5 (US). Again, there were no significant ($p > 0.05$) differences between the FCRs of Diet 4 (AL), 5 (US) and 7 (AS) but these values were significantly ($p < 0.05$) lower than the FCRs of Diet 2 (UL) and 3 (HL).

6.7.4. Protein Utilization

Protein utilization efficiency was measured in terms of protein efficiency ratio (PER) and apparent net protein utilization (ANPU %) as presented in Table 6.18. PERs followed similar trends to the FCRs with Diets 1 (Control) and 6 (HS) producing significantly the highest PERs. However, there were no significant ($p > 0.05$) differences between the

PERs of Diets 2 (UL) and 7 (AS) although these values were significantly ($p < 0.05$) higher than the PER of Diet 3 (HL).

Mean apparent net protein utilization (ANPU %) was calculated for all dietary treatments and ranged from 22.43 to 25.58 (Table 6.18). Both Diets 1 (Control) and 6 (HS) produced significantly ($p < 0.05$) higher ANPUs followed by Diet 4 (AL). There was no significant ($p > 0.05$) difference between the ANPUs of Diets 2 (UL) and 3 (HL) and these values were significantly ($p < 0.05$) lower than for the rest of the diets.

6.7.5. Apparent Nutrient Digestibility

Apparent nutrient digestibilities of the experimental diets were determined as described earlier (Section 2.8.1) and the results are presented in Table 6.19. Apparent dry matter digestibility values ranged from 65.10 to 77.62 with the Control diet having the highest value followed by Diet 6. Apparent protein digestibilities for all the experimental diets were fairly high ranging from 86.36% to 88.97% with the Control having the highest and Diet 2 (UL) the lowest. The apparent lipid, ash and energy digestibilities ranged from 87.60% to 93.09%, 29.88% to 39.34% and 73.45% to 82.08% respectively. Again the fish meal based Control diet had the highest lipid, ash and energy digestibility.

6.7.6. Carcass Composition

The results of proximate carcass composition of the fish at the start and at the end of the experiment are presented in Table 6.20. Fish from Diet 2 (UL) and 5 (US) had significantly ($p < 0.05$) the highest moisture

TABLE 6.19

Apparent nutrient digestibility of the experimental diets

	DIET NO.						
	1 (control)	2 (UL)	3 (HL)	4 (AL)	5 (US)	6 (HS)	7 (AS)
Apparent dry matter digestibility	77.62	65.10	66.66	67.10	65.77	67.54	67.33
Apparent protein digestibility	88.97	86.36	87.09	87.42	86.68	87.76	88.20
Apparent lipid digestibility	93.09	87.60	91.49	89.00	90.81	87.85	88.78
Apparent ash digestibility	39.34	29.88	33.50	34.01	31.26	32.79	30.41
Apparent energy digestibility	82.08	73.45	74.26	75.10	74.19	75.33	74.75

*No statistical analyses were possible as determinations were performed on pooled samples

TABLE 6.20

Proximate carcass composition (% fresh matter basis) of fish samples at the start and the end of the experiment

Components	INITIAL (all fish)	FINAL Diet No. 1 (Control)	2 (UL)	3 (HL)	4 (AL)	5 (US)	6 (HS)	7 (AS)	± S.E.[2]
Moisture	80.86	77.60^{d1}	78.47^{a}	78.00^{b}	77.91^{c}	78.53^{a}	77.70^{d}	78.04^{b}	0.05
Crude protein	13.18	14.77^{a}	14.23^{bc}	14.62^{a}	14.66^{a}	14.16^{c}	14.74^{a}	14.56^{a}	0.07
Crude lipid	3.27	5.18^{a}	4.48^{d}	4.64^{cd}	4.80^{bc}	4.48^{d}	4.91^{b}	4.71^{c}	0.06
Ash	2.29	2.31^{d}	2.50^{a}	2.44^{ab}	2.38^{bcd}	2.52^{a}	2.34^{cd}	2.40^{bc}	0.03
TOTAL	99.60	99.86	99.68	99.70	99.75	99.69	99.69	99.71	-

[1] Figures in the same row with the same superscripts are not significantly ($p > 0.05$) different

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

and lowest lipid content. Fish fed Diets 1 (Control) and 6 (HS) had significantly ($p < 0.05$) the lowest moisture content. However, there were no significant ($p > 0.05$) differences between carcass moisture contents of fish fed Diets 2 (UL) and 5 (US) and 3 (HL) and 7 (AS) respectively.

There were no significant ($p > 0.05$) differences between the carcass protein contents of fish fed Diets 1 (Control), 3 (HL), 4 (AL), 6 (HS), and 7 (AS) but these values were significantly higher than for the rest of the diets.

The Control diet produced significantly ($p < 0.05$) the highest carcass lipid content but there were no significant ($p > 0.05$) differences between the carcass lipid contents of fish fed Diet 4 (AL) and 6 (HAS) and 3 (HL), 4 (AL) and 7 (AS) respectively. Carcass ash contents ranged from 2.31 to 2.52.

6.7.7. General Health and Histological Examination

At the end of the experiment, no abnormalities or physical deformities were observed in fish examined from the various dietary treatment groups. Histological examination of various organs such as gills, liver, kidney, muscle, thyroid and intestine of fish from the various dietary treatments revealed no significant changes in these tissues.

6.8 DISCUSSION

Detoxification of linseed and sesame meals by heat and aqueous treatment

did not result in pronounced differences in proximate composition compared to untreated meals (Table 6.11). Similarly minor changes were found in the amino acid composition (Table 6.12). Mandokhot and Singh (1979) found small differences in proximate composition between untreated and two processed linseed meals.

Available lysine levels ranged from 0.96% to 1.06% (dry matter basis) or 80.00% to 82.81% of the total lysine (Table 6.13). In general, available lysine levels mirrored the relative pattern of total lysine values, broadly suggesting no serious effect of processing. Similarly Mandokhot and Singh (1979) reported no effect of processing on the amino acid composition, including available lysine content, of linseed meal. Abel et al. (1984) also found that available lysine content in full fat soybean was not affected by heat treatment. In contrast, a significant reduction (about 22%) in lysine content in rapeseed during heat processing was reported by Mustakas et al. (1965).

Heat treatment was found to be more effective than aqueous extraction in reduction of phytic acid content (Table 6.13) whilst the converse was true for hydrocyanic acid reduction. Lease (1966) found that four hour autoclaving only reduced the phytic acid content of sesame meal from 1.00% to 0.78%.

Although aqueous treatment was less effective in reducing phytic acid levels the reduction in linseed and sesame meals was appreciable (48.16% and 50.84% respectively). A 50% reduction in phytate content was reported (Anon, 1976, cited by Cheryan, 1980) when whole soybeans were

soaked in water for 24 hours at room temperature. At 55°C, about 90% of the phytate was removed. Chang et al. (1977) also reported that incubation of presoaked beans in water at 60°C for 10 hours lowered their phytate content by 90%. Joseph (1973) also observed a decrease in phytate in cassava as a result of soaking. The reduction in phytic acid observed after aqueous treatment was probably due to the breakdown or partial hydrolysis of the phytic acid.

Untreated linseed meal contained 0.032% HCN which was slightly lower than the reported value of 0.038% (Mani et al., 1949). Aqueous and heat treatment of linseed meal resulted in 34.38% and 53.12% reduction in HCN respectively. McGregor and McGinnis (1948) reported that simple water extraction of linseed meal at 25°C for 18 hours apparently destroyed the toxic factors and incorporation of treated meal at 30% in feed did not depress chick growth. Raymond et al. (1941) reported that boiling of cassava reduced the HCN content from 332 mg/kg to 10 mg/kg. Drying of cassava root at 60°C was capable of removing up to 90% of the HCN (Charavanapavan, 1944; cited by Coursey, 1973). The reduction of HCN in linseed meal by processing is likely to be due to volatilization of HCN (Liener, 1980).

The results of the present growth trial indicated that the use of detoxified linseed and sesame meals in carp diets improved the growth performances of fish. Apart from the Control, Diet 6 (HS) produced significantly ($p < 0.05$) the highest weight gain (Table 6.18) among the experimental diets followed by Diet 7 (AS) and 4 (AL). The weight gain of fish fed Diet 6 (HS) was about 95% of that obtained for fish fed the

fish meal based Control Diet.

Abel et al. (1984) reported higher growth performance of carp fed diets containing 50% heat treated soybean meal compared to that obtained with commercially available feeds. Lease (1966) reported that four hour autoclaving of sesame meal led to a significant increase in growth in chicks, no leg deformities and an increase in the zinc contents of the bone ash comparable to untreated meals with 60ppm of supplemental zinc. Since phytic acid is rather easily hydrolysed by autoclaving it seems likely that the beneficial effect of heat treatment resulted from this destruction of phytic acid (O'Dell et al., 1964). The improved growth performance of fish fed Diet 6 (HS) could be attributed to its lower phytic acid content (Table 6.15).

Among the linseed meal containing diets (2, 3 and 4) fish fed Diet 4 (AL) produced significantly ($p < 0.05$) the highest weight gain (Table 6.18), about 85% of the Control. Fish fed Diet 7 (AS) also produced higher weight gains than those fed Diet 5 (US). McGinnis and Polis (1946), Kratzer (1946, 1947) and McGregor and McGinnis (1948) reported increased growth in poultry fed "Water-treated" linseed meal compared to untreated meal. Singh and Punia (1979) also used water autoclaved linseed meal at 50% replacement of groundnut meal in poultry feed and found comparable growth to poultry fed groundnut meal.

No reports appear in the literature concerning the use of heat treated or aqueous treated linseed or sesame meal in fish feeds. However, the improved growth performance of fish fed Diets 4 (AL), 7 (AS) and 3 (HL)

compared to untreated meals is likely to be due to the lower HCN and phytic acid contents of these diets (Table 6.15).

The poor growth performance of fish fed Diet 2 (UL) was similar to that reported earlier in Experiment 1 (Section 3.3.5) but lower than that reported by Hasan (1986) who used the same level of linseed meal (25% of total protein) in carp diet and found growth similar to that of a fish meal based Control diet. These differences are probably due to the variation in chemical composition and anti-nutritional factors in the linseed meals used.

Use of detoxified linseed and sesame meals improved both food and protein utilization compared to untreated meals (Table 6.18). The food conversion ratio was lowest in Diet 6 (1.48) and increased in order of Diets 1 (1.50), 5 (1.53), 4 (1.54), 7 (1.56), 2 (1.60) and 3 (1.65). The FCRs, PERs and ANPUs obtained in this experiment are better than those obtained in Experiment 1 (Section 3.3.5) using the same inclusion levels of untreated linseed and sesame meals. However, the FCRs obtained with diets containing linseed (Diets 2, 3, 4) and sesame meals (Diets 5, 6, 7) in this trial are better than those of 2.33 (25% linseed protein diet) and 2.82 (25% sesame protein diet) observed by Hasan (1986).

Excluding the Control, all the test diets showed more or less similar dry matter digestibilities (Table 6.19) which are comparatively higher than those found in Experiment 1 (Section 3.3.8) but slightly lower than those found in Experiment 4.1 (Section 6.3.5). However, detoxification of meals slightly improved the dry matter digestibilities.

All the experimental diets showed fairly high apparent protein digestibilities ranging from 86.3% to 88.9%. As in Experiment 4.1 (Chapter 6), the detoxification of linseed and sesame meal did not improve the protein digestibility. Srivastava and Hill (1976) also reported that heat treatment did not improve the digestibility of rapeseed meals in rats.

The apparent energy digestibilities of the diets in the present study are similar to those obtained in Experiment 4.1 (Section 6.3.5). The fish meal based Control diet showed the highest (82.08%) energy digestibility (Table 6.19). The energy digestibility of the diets containing detoxified linseed and sesame meals are similar to the reported values for canola meal (75%) and dehulled soybean (75%) for trout (Hilton and Slinger, 1986 and Cho et al., 1982 respectively); peanut meal (76%) for channel catfish (Wilson and Poe, 1985), and somewhat higher than that for rapeseed meal (65%) and cotton seed meal (64%) for trout (Smith et al., 1980). However, detoxification slightly improved the energy digestibility.

Fish fed Diets 1 (Control) and 7 (HS) showed significantly ($p < 0.05$) the lowest carcass moisture and highest protein content (Table 6.20). The carcass protein contents of fish fed the rest of the diets showed small variations some of which were significant (Table 6.20). The inverse relationship between carcass lipid and moisture recorded here has also been reported by other workers (Andrews and Stickney, 1972; Atack et al., 1979). Fish fed diets containing detoxified meals showed slight increases in carcass lipid content which were still significantly lower

than that of Control fish. This lower carcass lipid content is well supported by the findings of Tacon and Ferns (1976) and Atack and Matty (1978) in rainbow trout fed single cell proteins and Appler (1985) in tilapia fed algal protein.

The results of the present investigation revealed that heat treatment was effective in destroying anti-nutritional factors in linseed and sesame meals. The use of detoxified meals in diets improved the growth performance and food utilization of carp compared to those fed untreated meals.

CHAPTER 7 : TOXICITY OF ANTI-NUTRIENTS IN PURIFIED DIETS FOR CARP - EXPERIMENT 5.1

Effect of phytic acid in purified diets containing varying levels of calcium and magnesium on growth and mineral availability in common carp (C. carpio L)

7.1 INTRODUCTION

The presence of phytic acid in many oilseed proteins such as soybeans, cottonseed, rapeseed, sesame and linseed is well documented (Erdman, 1979; Cheryan, 1980; Madhusudhan and Singh, 1983). Phytic acid is myoinositol, phosphorylated on all six hydroxyl groups and can bind ionically to protein in aqueous medium. Concern about its presence in food arises from the evidence that it affects the nutritional value of the product either by forming complexes with the protein or by reacting with calcium, magnesium, copper, zinc or iron in the food and thereby inhibiting the absorption of these important minerals (Hartman, 1979). O'Dell and Savage (1960) were the first workers to suggest that naturally occurring phytic acid in plant protein might reduce the availability of zinc.

Davies and Nitingale (1975) reported that inclusion of purified sodium phytate in a fibre-free rat diet, at a concentration which can commonly be encountered in practical soybean or cereal based diets, can fully account for the reduction of zinc availability when these natural phytate-rich diets are fed. The authors found that dietary phytate significantly reduced the average daily accumulation and whole body retention (relative to dietary intake) of iron, copper, manganese and zinc in rats whether or not the diet was supplemented with zinc.

O'Dell _et al._ (1958) found that the addition of excess calcium to a chick diet based on soybean protein (15ppm Zn) depressed growth rate unless it was supplemented with Zn. Excess calcium was also found to depress growth rate of swine in the presence of phytic acid but not in its

absence. In the absence of phytate, excess calcium had no detrimental effect, but when the diet contained phytate, excess calcium increased its zinc binding action (Oberleas et al., 1962).

The biological observation that an increase in the amount of Ca in diets of high phytate content aggravates a Zn deficiency in animals was investigated on a chemical basis of Zn-Ca-phytate interaction by Byrd and Matrone (1965). These authors reported that since Zn is a trace element it does not supply enough cations to form a significant precipitation of Zn-phytate. However, Ca cations increase the total cationic environment sufficiently to initiate co-precipitation with Zn to form insoluble phytates. Therefore, Zn in the presence of Ca would not be available for absorption and Zn deficiency would be aggravated.

On the other hand, when supplemental Zn is added to the diet, the concentration effect would at least partially overcome the Ca effect which has been observed biologically. Oberleas (1973) also reported that dietary phytate reduces the availability of Zn, Fe, Ca and Mg to mono-gastric animals by a mechanism involving precipitation of metal-phytate complexes within the intestinal lumen.

The effects of phytic acid (either naturally occurring or synthetic) on growth and mineral bioavailability in rats, poultry and swine have been shown conclusively to be adverse especially when high dietary levels of calcium have been employed.

Reports, so far, on the effects of phytic acid on mineral bioavailability in fish are controversial. Spinelli et al. (1983) found that rainbow trout fed diets containing phytic acid had 10% reduced growth and feed conversion. Increasing Ca and Mg content of the diets in the presence of phytic acid did not affect growth and feed conversion. The Zn and Fe levels in the blood of fish fed diets containing phytic acid were not significantly different from the Control. It was concluded that the reduced growth in fish fed diets containing phytic acid was related to protein availability rather than to an alteration in the bioavailability of Zn, Fe or Ca.

In contrast, Richardson et al. (1985) reported that high dietary phytic acid concentration (2.58%) depressed chinook salmon growth, food and protein conversion (PER) and thyroid function and increased mortality. Moreover, high dietary levels of calcium (4.8%-5.1%), coupled with phosphorous, significantly impaired the growth and appetite of low and high phytic acid groups. Plasma Zn levels were directly related to dietary Zn concentration and inversely related to dietary phytic acid level.

In previous experiments (Chapters 3, 4 and 6), carp fed diets containing linseed and sesame meals produced poor growth performance compared to the Control. Phytic acid is the principal anti-nutritional factor in both linseed and sesame meal. The controversy in the literature about the effects of phytic acid on mineral bioavailability led to the present study. Phytic acid in the form of sodium phytate, was included in purified diets to investigate

(1) whether synthetic phytic acid acts in the same way as naturally occurring phytates, and

(2) the effects of dietary phytic acid on growth and mineral bioavailability in carp fed diets containing varying levels of dietary calcium and magnesium.

7.2 MATERIALS AND METHODS

7.2.1 Experimental Systems and Animals

The experimental systems used in the present study are described in detail in Sections 2.1.1 and 2.1.2. The sources of experimental animals and their quarantine procedures are described in Section 2.2.1.

7.2.2. Analytical Techniques

Proximate composition, chromic oxide, amino acids, total energy of experimental diets, faeces and fish carcasses were determined as described in the appropriate sections of Chapter 2. The amino acid tryptophan could not be analysed because of its destruction during acid hyrolysis. Mineral composition of feeds, fish carcasses, livers and kidneys of fish fed the experimental diets were determined as described in Section 2.6.1.

At the end of the experiment, fish were anaesthetised with benzocaine solution (Ross and Geddes, 1979) and blood was drawn from the caudal vessels with preheparinized syringes. Following centrifugation the

plasma from fish from each replicate was pooled and stored at -20°C. Plasma mineral concentrations were analysed using a Perkin Elmer 2280 Atomic Absorption Spectrophotometer. Phytic acid content of the experimental diets was determined as described earlier (Section 2.7.1).

7.2.3. Diet Formulation and Preparation

Nine purified and two semi-purified isonitrogenous diets were formulated using vitamin-free casein and sesame meal as protein sources. The specifications of the experimental diets are shown in Table 7.1. Both casein and gelatin were obtained from Sigma Chemical Company Ltd, Poole, Dorset, England. The sesame meal used was of Bangladeshi origin.

All diets were formulated to contain 40% protein and 10% lipid (Table 7.2). Phytic acid was added at two different levels (0.5% and 1.0%). In purified diets sodium phytate (Sigma Chemical Co Ltd) was used as a dietary phytic acid source. Although sodium phytate contained about 22% sodium, dietary levels of sodium did not exceed those found normally in commercial fish feeds (Tacon and De Silva, 1983).

Phytic acid in Diets 10 and 11 was supplied by naturally occurring phytic acid in sesame meal. Sesame meal was used as a phytic acid source to study whether there was any difference in growth responses of fish on diets containing synthetic and natural phytic acid.

The varying levels of calcium and magnesium in the diets were maintained by using two different Ca-Mg salt mixtures the compositions of which are shown in Table 7.1.

TABLE 7.1

Specification of the experimental diets used to study the effect of phytic acid on the growth of carp

DIET NO.	SPECIFICATION
1.	Control (Casein + Gelatin)
2.	Control + Ca-Mg mix - 1
3.	Control + Ca-Mg mix - 2
4.	Control + 0.5% phytic acid
5.	Control + 0.5% phytic acid + Ca-Mg mix -1
6.	Control + 0.5% phytic acid + Ca-Mg mix -2
7.	Control + 1.0% phytic acid
8.	Control + 1.0% phytic acid + Ca-Mg mix - 1
9.	Control + 1.0% phytic acid + Ca-Mg mix - 2
10.	Sesame diet - containing 0.5% phytic acid
11.	Sesame diet - containing 1.0% phytic acid

Composition of Ca-Mg mix:

Ca-Mg mix 1 : 2.460g of $CaHPO4,2H_2O$ and 0.5067g of $MgSO4.7H_2O$

Ca-Mg mix 2 : 5.675g of $CaHPO4,2H_2O$ and 1.0134g of $MgSO4,7H_2O$

TABLE 7.2

Formulation of experimental diets used in Experiment 5.1

Ingredients	DIET NO.										
	1	2	3	4	5	6	7	8	9	10	11
Casein	31.75	31.75	31.75	31.75	31.75	31.75	31.75	31.75	31.75	34.39	26.48
Gelatin	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	-	-
Sesame meal	-	-	-	-	-	-	-	-	-	20.40	40.80
Cod liver oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	4.00
Vitamin premix[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Binder (CMC)[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Dextrin	29.00	28.00	25.00	29.00	28.00	25.00	29.00	27.00	25.00	20.00	15.22
α-cellulose	10.75	8.78	8.06	9.98	8.01	7.29	9.20	8.23	6.51	6.71	-
Ca-Mg mix - 1	-	2.97	-	-	2.97	-	-	2.97	-	-	-
Ca-Mg mix - 2	-	-	6.69	-	-	6.69	-	-	6.69	-	-
Sodium phytate[3]	-	-	-	0.77	0.77	0.77	1.55	1.55	1.55	-	-
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1] For composition of mineral and vitamin premixes see Tables 2.3 and 2.4 respectively

[2] Sodium carboxymethyl cellulose (high viscosity)

[3] Source of phytic acid

TABLE 7.3

Analysed composition of the experimental diets used in Experiment 5.1
(% dry matter basis unless otherwise stated)

Components	DIET NO.										
	1	2	3	4	5	6	7	8	9	10	11
Dry matter	93.50	93.00	93.36	93.65	94.53	92.15	94.23	92.99	93.52	95.00	94.55
Crude protein	40.38	40.34	41.50	41.08	41.00	41.22	41.07	40.80	40.92	40.10	39.98
Crude lipid	9.81	9.73	10.06	9.88	10.12	10.08	9.86	9.78	9.92	10.20	10.12
Ash	4.48	6.73	7.56	4.60	7.16	9.94	5.48	7.52	9.54	6.92	9.19
Crude fibre	8.27	6.56	6.23	7.62	6.30	6.00	8.06	6.52	6.11	9.73	9.25
NFE[1]	37.06	36.64	34.65	36.82	35.42	32.76	35.53	35.38	33.51	33.05	31.46
Chromic oxide	0.48	0.49	0.50	0.49	0.50	0.49	0.48	0.49	0.48	0.49	0.50
Total energy (Kcal/g)	4.60	4.58	4.56	4.61	4.57	4.53	4.54	4.55	4.49	4.52	4.50
PE Ratio[2]	87.78	88.04	91.00	89.11	89.71	90.99	90.46	89.67	91.13	88.71	88.84
Phytic acid	-	-	-	0.49	0.50	0.48	1.00	1.00	1.01	0.48	0.98

[1] Nitrogen free extractive calculated as

100 - (Moisture+Crude protein+Crude lipid+Ash+Crude fibre)

[2] Protein energy ratio in mg protein/Kcal of total energy

TABLE 7.4

Mineral composition of the test diets used in Experiment 5.1
(dry matter basis)

Minerals	DIET NO.										
	1	2	3	4	5	6	7	8	9	10	11
g/kg											
Ca	9.25	15.12	22.15	9.16	14.96	22.07	9.27	14.87	22.29	12.99	16.01
P	8.06	8.10	8.16	9.41	9.46	9.49	10.81	10.89	10.95	10.95	13.50
Mg	0.58	1.08	1.60	0.59	1.09	1.59	0.59	1.09	1.61	1.03	2.47
Na	0.98	0.99	0.97	1.17	1.18	1.17	1.37	1.40	1.39	1.03	1.10
K	1.08	1.10	1.09	1.07	1.08	1.06	1.10	1.08	1.07	1.15	1.22
mg/kg											
Fe	204	206	203	207	205	208	209	210	206	220	235
Zn	58	60	59	61	57	58	59	60	61	65	72
Cu	9.20	9.24	9.26	9.32	9.23	9.21	9.36	9.24	9.28	9.45	9.66
Mn	26.31	27.00	26.50	24.42	26.80	26.71	26.62	26.56	26.72	27.10	27.40

TABLE 7.5

Amino acid composition of the test diets used in Experiment 5.1

(% dry matter basis)

Amino acids	DIET NO.											Requirement for carp[1]
	1	2	3	4	5	6	7	8	9	10	11	
Arginine	1.96	1.98	2.00	1.95	1.97	2.02	2.01	1.98	1.99	1.94	2.15	1.52
Histidine[2]	-	-	-	-	-	-	-	-	-	-	-	0.56
Isoleucine	1.83	1.78	1.85	1.84	1.81	1.88	1.87	1.84	1.89	1.83	1.71	0.92
Leucine	2.85	2.82	2.80	2.86	2.83	2.82	2.89	2.90	2.91	3.03	3.06	1.64
Lysine	3.23	3.22	3.24	3.32	3.25	3.24	3.32	3.33	3.31	1.80	1.78	2.12
Methionine	0.74	0.78	0.70	0.73	0.72	0.73	0.75	0.77	0.77	0.57	0.62	0.64
Cystine	0.18	0.19	0.17	0.18	0.18	0.17	0.17	0.18	0.17	0.16	0.31	-
Phenylalanine	1.71	1.70	1.74	1.75	1.72	1.72	1.66	1.69	1.68	1.89	1.71	1.16
Tyrosine	1.42	1.42	1.41	1.45	1.39	1.44	1.42	1.39	1.40	1.51	1.38	-
Threonine	1.30	1.36	1.31	1.38	1.37	1.33	1.33	1.35	1.33	1.50	1.44	1.32
Valine	2.05	2.08	2.06	2.04	2.10	2.05	2.06	2.09	2.07	2.01	2.06	1.16
Alanine	1.81	1.82	1.80	1.84	1.84	1.82	1.81	1.75	1.76	1.39	1.41	-
Aspartic acid	3.10	3.07	3.02	3.08	3.03	3.07	2.97	2.99	2.99	2.71	2.69	-
Glutamic acid	7.62	7.68	7.66	7.72	7.62	7.65	7.68	7.75	7.69	8.28	7.80	-
Glycine	2.71	2.81	2.84	2.81	2.79	2.79	2.83	2.82	2.76	2.18	2.29	-
Proline	4.60	4.63	4.65	4.63	4.61	4.60	4.60	4.65	4.61	3.61	3.54	-
Serine	1.84	1.84	1.79	1.75	1.80	1.83	1.74	1.83	1.80	1.91	1.87	-

[1]Data for essential amino acid requirement of carp from Ogino (1980)

[2]No data as peak not differentiated in the chromatogram

TABLE 7.6

A summary of the methodology used to study the effect of phytic acid in purified diets containing varying levels of calcium and magnesium on the growth of common carp

Fish	C. carpio, average initial weight 4.20 ± 0.04g
Duration of Experiment	Eight weeks
Treatments	Effect of varying levels of dietary phytic acid, calcium and magnesium on the growth of common carp
No. of Treatment	11
Replication	3/treatment
Water Temperature	27° ± 1°C
Stocking Density	12/tank
Water Flow Rate	1 litre/min/tank
Carcass Sampling	Initial sample - 12 fish at the start of the experiment Final sample - 15 fish per treatment (4 from each replicate)
Faeces Collection	Collected twice daily for two weeks, dried at 60°C in an oven and used for subsequent chemical analysis for protein digestibility study
Physico-chemical characteristics of water monitored	Temperature, pH, dissolved oxygen and total ammonia

Feed preparation was as described in Section 2.2.5. All diets were analysed for proximate, mineral and amino acid composition and the results are presented in Tables 7.3, 7.4 and 7.5 respectively. Phytic acid contents in the diets were analysed and are shown in Table 7.3.

7.2.4. General Experimental Procedure

Each treatment had three replicates, 12 fish per replicate, with mean initial weights of 4.15g-4.26g. The acclimation and weighing procedures were as described in Section 2.2.3. Fish were bulk weighed weekly after 12 hour starvation during the experimental period. The experiment was conducted for a period of eight weeks. A summary of the methodology used in the present experiment is given in Table 7.6.

7.2.5. Feeding Rates

Fish were transferred to the faecal collection system (see Section 2.1.2) at the beginning of the eighth week. Faeces were collected twice daily - morning and evening for one week. Details of faecal collection procedures are described in Section 3.2.6. Collected faeces were dried in an oven at 60°C. Faecal samples of each replicate tank were pooled to represent respective treatments and kept in air-tight containers for subsequent chemical analysis.

7.2.7. Histological Techniques

Histological techniques were as described in Section 3.2.7. Fifteen fish per treatment were sampled for histopathology to assess changes in gills, thyroid, liver muscle, kidney and intestine.

7.2.8. Analysis of Experimental Data

Experimental results were analysed as described in Section 2.8.1. Statistical analyses were performed as described in Section 2.9.1.

7.3 RESULTS

7.3.1. Analysed Composition of the Diets

Proximate composition of the experimental diets is shown in Table 7.3. Moisture contents varied between 5.00% and 7.01%. Protein levels were similar and ranged between 39.98% and 41.50%.

Lipid and energy contents showed little variation between diets. Ash contents increased with the incorporation of salt mixtures in the respective diets. Crude fibre contents in Diets 10 and 11 were slightly higher than for the rest of the diets due to the crude fibre content (about 23%) of the sesame meal used.

Calcium, magnesium and sodium contents in the diets (Table 7.4) varied in accordance with the inclusion of salt mixtures and sodium phytate. However, the mineral contents were within the normal ranges found in commercial fish feeds .(Tacon and De Silva, 1983). The amino acid compositions of the experimental diets are presented in Table 7.5. Diets 10 and 11 were slightly deficient in lysine and methionine.

7.3.2. Growth

The growth responses of carp fed the various experimental diets are presented as initial and final mean weight, weight gain and specific

growth rate (SGR) in Table 7.7 and graphically in Figure 7.1. It appears that growth responses were significantly affected by the inclusion of dietary phytic acid.

Diet 1 (Control) produced significantly ($p < 0.05$) the best growth response throughout the experimental period, while Diet 9 (1% phytic acid and Ca-Mg salt mix-2) resulted in the poorest growth (Table 7.7). All the diets containing various levels of phytic acids produced significantly ($p < 0.05$) lower weight gains than the Control. Weight gains were lowest when diets with phytic acid also contained higher levels of calcium and magnesium. However, there were no significant ($p > 0.05$) differences between the growth responses of fish fed diets containing synthetic (Diet 4) and natural (Diet 10) phytic acid.

SGRs for the different treatment groups ranged from 2.62 to 3.30. The SGRs for the Control and Diet 2 were significantly ($p < 0.05$) higher than for the rest of the diets. There was no significant ($p > 0.05$) difference in SGR between Diet 4 (containing synthetic phytic acid) and 10 (containing natural phytic acid). Both Diets 4 and 10 produced SGRs about 95% of that for fish fed the Control diet.

7.3.3. Food Conversion Ratio (FCR)

Mean food conversion ratios (FCRs) are presented in Table 7.7. Diets 1 and 2 produced significantly ($p < 0.05$) the lowest (best) FCRs. There were no significant ($p > 0.05$) differences between FCRs of Diets 1 and 2, 4 and 10, 5 and 6 and 5 and 10 respectively. FCRs ranged from 1.51 to 1.96 and Diet 9 had significantly ($p < 0.05$) the highest (poorest) FCR.

TABLE 7.7

Growth and food utilization of common carp fed the experimental diets for 8 weeks

Components	DIET NO.											± S.E.[2]
	1 (Control)	2	3	4	5	6	7	8	9	10	11	
Mean initial weight (g)	4.15^{a1}	4.17^{a}	4.18^{a}	4.23^{a}	4.23^{a}	4.26^{a}	4.24^{a}	4.16^{a}	4.19^{a}	4.20^{a}	4.21^{a}	0.04
Mean final weight (g)	26.27^{a}	26.17^{a}	25.20^{b}	24.44^{c}	23.50^{d}	22.70^{e}	21.80^{f}	20.92^{g}	18.20^{i}	24.21^{c}	20.42^{h}	0.09
Weight gain (g)	22.12^{a}	22.00^{a}	21.02^{b}	20.21^{c}	19.26^{d}	18.44^{e}	17.57^{f}	16.76^{g}	14.01^{i}	20.01^{c}	16.21^{h}	0.06
% weight gain	533^{a}	528^{a}	503^{b}	477^{c}	455^{d}	433^{e}	415^{f}	403^{g}	334^{i}	477^{c}	385^{h}	3.27
SGR (% day)	3.30^{a}	3.28^{a}	3.21^{b}	3.13^{c}	3.06^{d}	2.99^{e}	2.93^{f}	2.88^{g}	2.62^{i}	3.13^{c}	2.82^{h}	0.01
SGR as % of Control	100	99.4	97.3	94.8	92.3	90.6	88.8	87.3	79.4	94.8	85.4	-
FCR	1.51^{h}	1.51^{h}	1.54^{g}	1.59^{f}	1.64^{de}	1.67^{d}	1.75^{c}	1.77^{c}	1.96^{a}	1.61^{ef}	1.83^{b}	0.01
PER	1.63^{a}	1.64^{a}	1.54^{b}	1.53^{b}	1.49^{c}	1.45^{d}	1.39^{e}	1.38^{e}	1.25^{f}	1.55^{b}	1.37^{e}	0.01
ANPU (%)	24.58^{a}	24.68^{a}	23.20^{b}	22.75^{c}	21.94^{d}	21.28^{e}	20.23^{f}	19.91^{f}	17.86^{g}	23.06^{bc}	20.00^{f}	0.11
Apparent protein digestibility	93.14	93.20	93.12	91.82	91.44	90.86	88.75	88.28	87.12	90.64	87.24	-

[1] Figures in the same row having the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

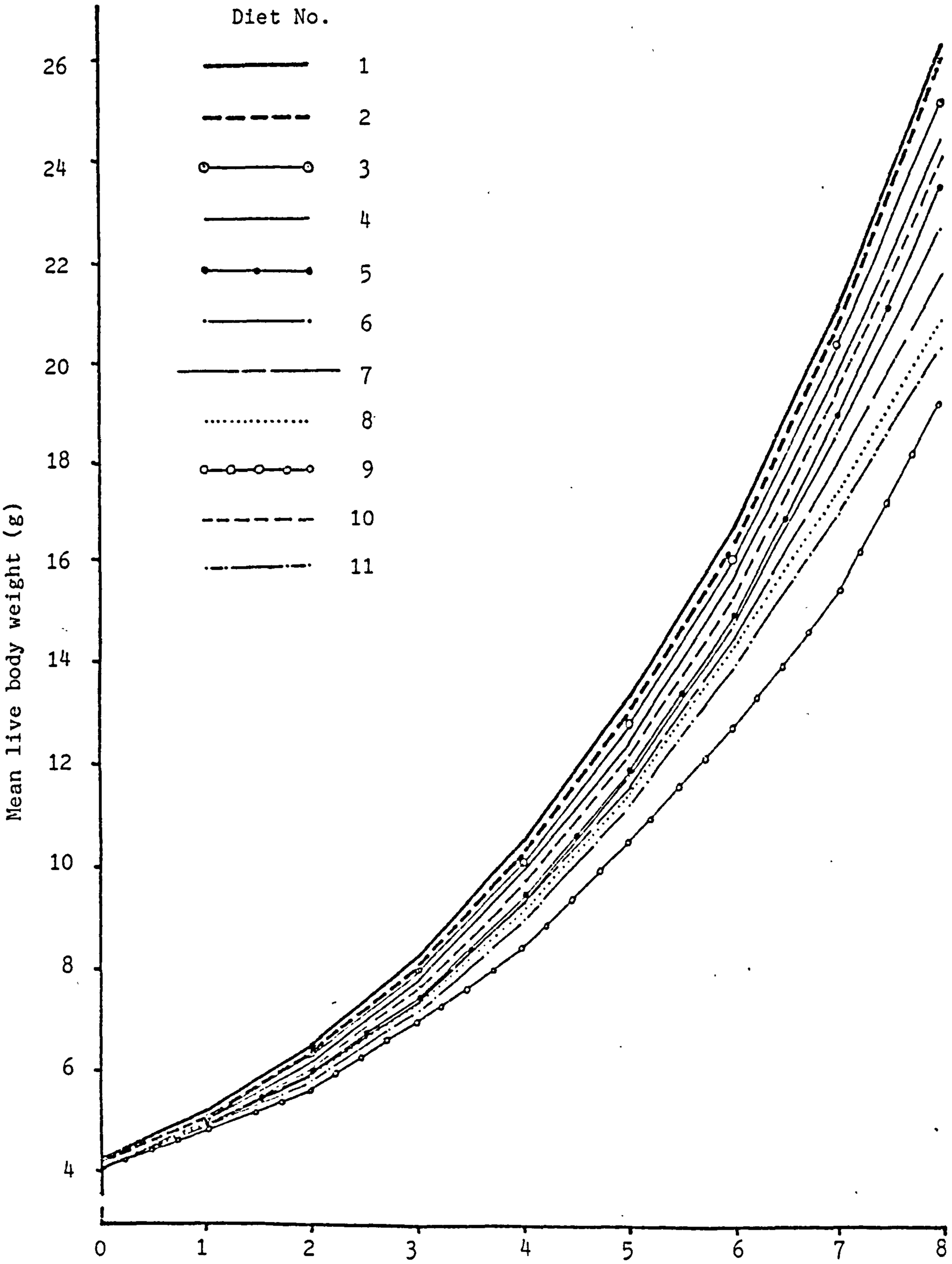


FIGURE 7.1 Growth responses of fish fed experimental diets for 8 weeks

7.3.4. Protein Utilization

Protein utilization was measured in terms of protein efficiency ratio (PER), apparent net protein utilization (ANPU %) and apparent protein digestibility (Table 7.7). PERs followed similar trends to FCRs with Diets 1 and 2 producing significantly ($p < 0.05$) the highest PERs. However, there were no significant ($p > 0.05$) differences between PERs of Diets 3, 4 and 10 but these values were significantly ($p < 0.05$) higher than for the other diets (5, 6, 7, 8, 9 and 11). PERs ranged from 1.37 to 1.64.

Mean apparent net protein utilization (ANPU %) was calculated for all dietary treatments and ranged from 17.86 to 24.68 (Table 7.7). Diets 1 and 2 produced significantly ($p < 0.05$) the highest ANPU. There was no significant ($p > 0.05$) difference between the ANPUs of Diets 3 and 10 but these values were significantly higher than for the rest of the diets. All the diets showed fairly high apparent protein digestibilities ranging from 87.12% to 93.20% with Diet 1 having the highest and Diet 9 the lowest.

7.3.5. Plasma Mineral Concentrations

The mean plasma mineral concentration (mg/l) of the fish at the end of the experiment is shown in Table 7.8. Dietary treatment significantly affected plasma levels of calcium, zinc and iron but not levels of magnesium, sodium, potassium and copper. Plasma calcium levels ranged between 14.49 and 28.36 mg/l. High dietary calcium and magnesium

TABLE 7.8

Final mean plasma concentration (mg/l) in carp fed the experimental diets

Minerals	DIET NO.											± S.E.[2]
	1	2	3	4	5	6	7	8	9	10	11	
Ca	102.12^{abc1}	101.58^{abc}	104.81^{a}	102.01^{abc}	102.85^{ab}	100.03^{abc}	96.44^{bcd}	96.21^{cd}	92.10^{d}	102.20^{abc}	92.89^{d}	1.96
Mg	20.11^{a}	20.45^{a}	22.29^{a}	20.75	21.26	20.30	18.47	17.27	16.19	20.26	17.36	1.32
Na	2647^{a}	2671^{a}	2455^{a}	2366^{a}	2536^{a}	2599^{a}	2355^{a}	2376^{a}	2453^{a}	2505^{a}	2398^{a}	102
K	93.04^{a}	94.21^{a}	95.34^{a}	96.23^{a}	93.39^{a}	97.00^{a}	93.53^{a}	93.91^{a}	95.03^{a}	93.32^{a}	94.65^{a}	1.21
Zn	27.78^{a}	28.36^{a}	27.31^{a}	25.09^{ab}	22.32^{bc}	23.37^{bc}	19.78^{cd}	16.77^{de}	14.49^{e}	25.17^{ab}	15.82^{de}	1.30
Fe	5.11^{de}	5.34^{bcd}	5.62^{ab}	4.98^{e}	5.39^{abcd}	5.48^{abc}	5.10^{de}	5.71^{a}	5.13^{de}	5.60^{ab}	5.20^{cde}	0.097
Cu	0.89^{a}	0.92^{a}	0.91^{a}	0.92^{a}	0.88^{a}	0.90^{a}	0.92^{a}	0.93^{a}	0.88^{a}	0.94^{a}	0.89^{a}	0.02

[1] Figures in the same row having the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of mean calculated from the residual mean square in the analysis of variance

TABLE 7.9

Final mean liver mineral content (mg/kg, fresh matter basis) of carp fed the experimental diets

Minerals	DIET NO.											± S.E.[2]
	1	2	3	4	5	6	7	8	9	10	11	
Ca	510.2^{a1}	498.1^{ab}	503.6^{ab}	480.4^{b}	488.3^{ab}	480.1^{b}	450.3^{c}	438.6^{c}	434.1^{c}	490.7^{ab}	448.4^{c}	8.46
Mg	98.3^{a}	101.4^{a}	102.2^{a}	95.3^{abc}	92.4^{abc}	92.1^{abc}	82.1^{cd}	78.8^{d}	74.5^{d}	96.2^{ab}	83.3^{bcd}	4.02
Zn	88.32^{a}	86.70^{ab}	89.27^{a}	85.44^{abc}	82.30^{abc}	77.40^{bc}	75.34^{c}	58.36^{d}	53.37^{d}	54.56^{d}	51.41^{d}	3.19
Fe	85.36^{a}	88.42^{a}	90.48^{a}	94.22^{a}	87.73^{a}	84.54^{a}	83.66^{a}	80.43^{a}	80.46^{a}	88.83^{a}	84.43^{a}	3.39
Cu	16.26^{a}	16.80^{a}	16.57^{a}	14.05^{ab}	12.67^{bc}	12.10^{bcd}	10.62^{cd}	10.35^{cd}	9.61^{d}	14.25^{ab}	10.65^{cd}	0.82

[1] Figures in the same row having the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of mean calculated from the residual mean square in the analysis of variance

TABLE 7.10

Final mean kidney mineral content (mg/kg, fresh matter basis) of carp fed the experimental diets

Minerals	DIET NO.											± S.E.[2]
	1	2	3	4	5	6	7	8	9	10	11	
Ca	646.9^{a1}	629.3^{abc}	636.6^{ab}	609.1^{abc}	614.9^{abc}	588.1^{bc}	600.1^{abc}	572.8^{c}	566.1^{c}	632.0^{ab}	585.4^{bc}	15.77
Mg	120.7^{abc}	123.7^{a}	126.5^{a}	121.3^{ab}	122.9^{a}	106.5^{bcd}	118.6^{abc}	105.2^{cd}	98.5^{d}	125.4^{a}	102.3^{d}	4.77
Zn	448.6^{ab}	457.1^{ab}	464.5^{a}	430.1^{bcd}	438.1^{abc}	416.5^{cd}	403.4^{d}	360.1^{e}	328.1^{f}	456.2^{ab}	362.8^{e}	9.00
Fe	131.4^{abc}	123.1^{bc}	131.7^{abc}	119.7^{d}	131.1^{abc}	120.1^{bc}	141.9^{a}	133.01^{ab}	137.3^{a}	132.4^{abc}	129.7^{abc}	3.86
Cu	4.01^{a}	3.51^{c}	3.94^{ab}	3.92^{ab}	3.71^{bc}	3.70^{bc}	3.55^{c}	3.50^{c}	4.02^{a}	3.96^{ab}	3.49^{c}	0.09

[1] Figures in the same row having the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

contents had no influence on plasma zinc unless the diet simultaneously contained phytic acid. Plasma iron levels ranged from 5.11 to 5.71 mg/l.

7.3.6. Liver Mineral Contents

Dietary treatment significantly affected liver calcium, magnesium, zinc and copper content but not iron content (Table 7.9). Liver calcium content in different dietary groups ranged between 434.1 and 510.2 mg/kg. There were no significant ($p > 0.05$) differences between liver calcium contents of fish fed Diets 1, 2, 3, 5 and 10 but these values were significantly ($p < 0.05$) higher than for the rest of the diets. Liver magnesium, zinc and copper in the different treatment groups ranged from 74.5 to 102.2, 51.41 to 89.27 and 9.61 to 16.80 mg/kg respectively (Table 7.9).

7.3.7. Kidney Mineral Contents

Dietary treatment significantly affected the kidney mineral contents in different treatment groups (Table 7.10). However, there were no significant ($p > 0.05$) differences between kidney calcium and magnesium contents of fish fed Diets 1, 2, 3, 4, 5 and 10 but these values were significantly ($p < 0.05$) higher than for the rest of the diets. Similarly there were no significant differences between kidney zinc contents of fish fed Diets 1, 2, 3, 5 and 10 but these values were significantly ($p < 0.05$) higher than for the rest of the diets. The higher calcium and magnesium content in diets had little influence on kidney mineral contents except when the diets contained phytic acid. Kidney iron and

copper contents of fish fed the experimental diets ranged from 119.7 to 141.9 and 3.49 to 4.02 mg/kg respectively.

7.3.8. Carcass Composition

The results of proximate carcass analysis of fish at the start and at the end of the experiment are presented in Table 7.11. Fish fed Diets 1, 2 and 3 had significantly ($p < 0.05$) the lowest moisture and highest protein and lipid content. There were no significant ($p > 0.05$) differences between moisture, crude protein, crude lipid and ash content of fish fed Diets 4 and 10. Fish fed diets (8 and 9) containing higher levels of dietary phytic acid, calcium and magnesium had significantly ($p < 0.05$) the lowest carcass protein and lipid content. The carcass ash content ranged between 2.84% to 3.04%.

7.3.9. Carcass Mineral Content

Carcass mineral composition of fish fed the experimental diets is presented in Table 7.12. Dietary phytic acid and higher levels of calcium and magnesium had no influence on the retention of sodium, potassium, iron, copper and manganese. However, carcass calcium magnesium, phosphorous and zinc contents were significantly influenced by the dietary phytic acid, particularly when the diets simultaneously contained higher levels of calcium and magnesium.

7.3.10. General Health and Histological Examination

A pathological examination of 15 fish from each treatment group at the end of the experiment revealed no evidence of bacterial or viral

TABLE 7.11

Proximate carcass composition analysis (% fresh weight basis) of fish at the start and the end of the experiment

Components	Initial (all fish)	Final DIET NO.											± S.E.[2]
		1	2	3	4	5	6	7	8	9	10	11	
Moisture	80.46	77.12^{gl}	77.07^{g}	77.24^{g}	77.83^{f}	78.31^{e}	78.35^{de}	78.60^{c}	78.84^{b}	79.38^{a}	77.78^{f}	78.49^{cd}	0.06
Crude protein	13.34	14.80^{a}	14.80^{a}	14.76^{ab}	14.63^{bc}	14.53^{cd}	14.42^{de}	14.33^{ef}	14.19^{fg}	14.09^{g}	14.63^{bc}	14.38^{de}	0.05
Crude lipid	3.28	5.06^{a}	4.98^{ab}	4.89^{b}	4.22^{cd}	4.15^{d}	3.93^{e}	3.85^{e}	3.69^{f}	3.36^{g}	4.28^{c}	3.87^{e}	0.03
Ash	2.52	2.92^{bcde}	2.99^{abc}	3.04^{a}	2.90^{cde}	2.94^{abcd}	3.00^{ab}	2.85^{de}	2.87^{de}	2.86^{de}	2.88^{de}	2.84^{e}	0.03
TOTAL	99.60	99.90	99.84	99.93	99.58	99.93	99.70	99.63	99.59	99.69	99.57	99.58	-

[1] Figures in the same row having the same superscripts are not significantly different ($p > 0.05$)

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

TABLE 7.12

Final mean carcass mineral composition (fresh matter basis) of carp fed experimental diets

Minerals	DIET NO.											± S.E.[2]
	1	2	3	4	5	6	7	8	9	10	11	
Ca (g/kg)	6.30^{bl}	6.41^{a}	6.46^{a}	6.26^{b}	6.24^{b}	6.21^{bc}	6.11^{c}	5.99^{d}	5.80^{e}	6.23^{b}	6.00^{d}	0.03
Mg "	0.26^{abc}	0.27^{ab}	0.29^{a}	0.25^{abc}	0.25^{abc}	0.25^{abc}	0.22^{bcd}	0.20^{cd}	0.18^{d}	0.26^{abc}	0.23^{abcd}	0.02
P "	4.80^{a}	4.81^{a}	4.82^{a}	4.85^{a}	4.83^{a}	4.87^{a}	4.46^{b}	4.29^{c}	4.19^{d}	4.50^{b}	4.22^{cd}	0.03
Na "	0.99^{a}	1.05^{a}	1.00^{a}	1.02^{a}	1.05^{a}	1.06^{a}	1.02^{a}	1.03^{a}	1.00^{a}	0.99^{a}	1.02^{a}	0.02
K "	2.32^{a}	2.29^{a}	2.31^{a}	2.31^{a}	2.34^{a}	2.37^{a}	2.30^{a}	2.28^{a}	2.27^{a}	2.32^{a}	2.30^{a}	0.03
Fe (mg/kg)	22.01^{a}	21.04^{a}	23.11^{a}	22.98^{a}	21.96^{a}	21.23^{a}	21.14^{a}	20.58^{a}	19.89^{a}	22.00^{a}	20.77^{a}	0.94
Cu "	1.30^{a}	1.32^{a}	1.29^{a}	1.34^{a}	1.32^{a}	1.30^{a}	1.32^{a}	1.28^{a}	1.29^{a}	1.32^{a}	1.29^{a}	0.02
Zn "	80.22^{a}	78.66^{a}	79.29^{a}	77.25^{a}	76.32^{a}	74.41^{a}	65.54^{b}	54.01^{c}	44.73^{d}	75.62^{a}	54.26^{c}	2.19
Mn "	0.70^{a}	0.69^{a}	0.72^{a}	0.70^{a}	0.69^{a}	0.71^{a}	0.70^{a}	0.69^{a}	0.72^{a}	0.70^{a}	0.69^{a}	0.04

[1]Figures in the same row having the same superscripts are not significantly different ($p > 0.05$)

[2]Standard error of treatment mean calculated from the residual mean square in the analysis of variance

PLATE 7.1.a

Section of intestine of carp fed diets containing high phytic acid, calcium and magnesium content (Diet 9) from Experiment 5.1. Note marked hypertrophy and vacuolization of the cytoplasm.

(x250)

PLATE 7.1.b

Section of intestine of carp fed casein and gelatin based Control diet from Experiment 5.1. Note normal columnar shaped cells with fine granular cytoplasm.

(x250)

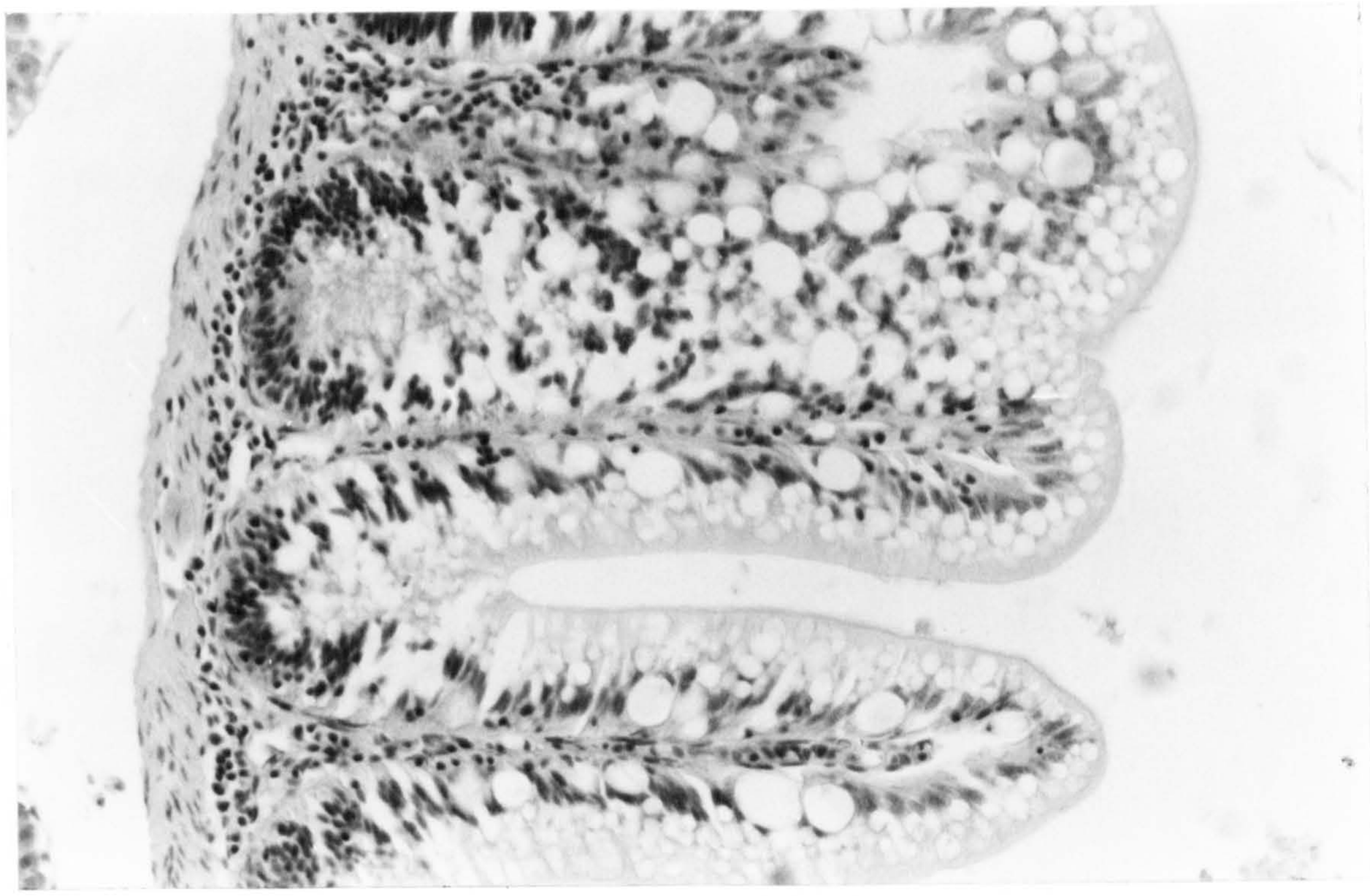


Plate 7.1.a

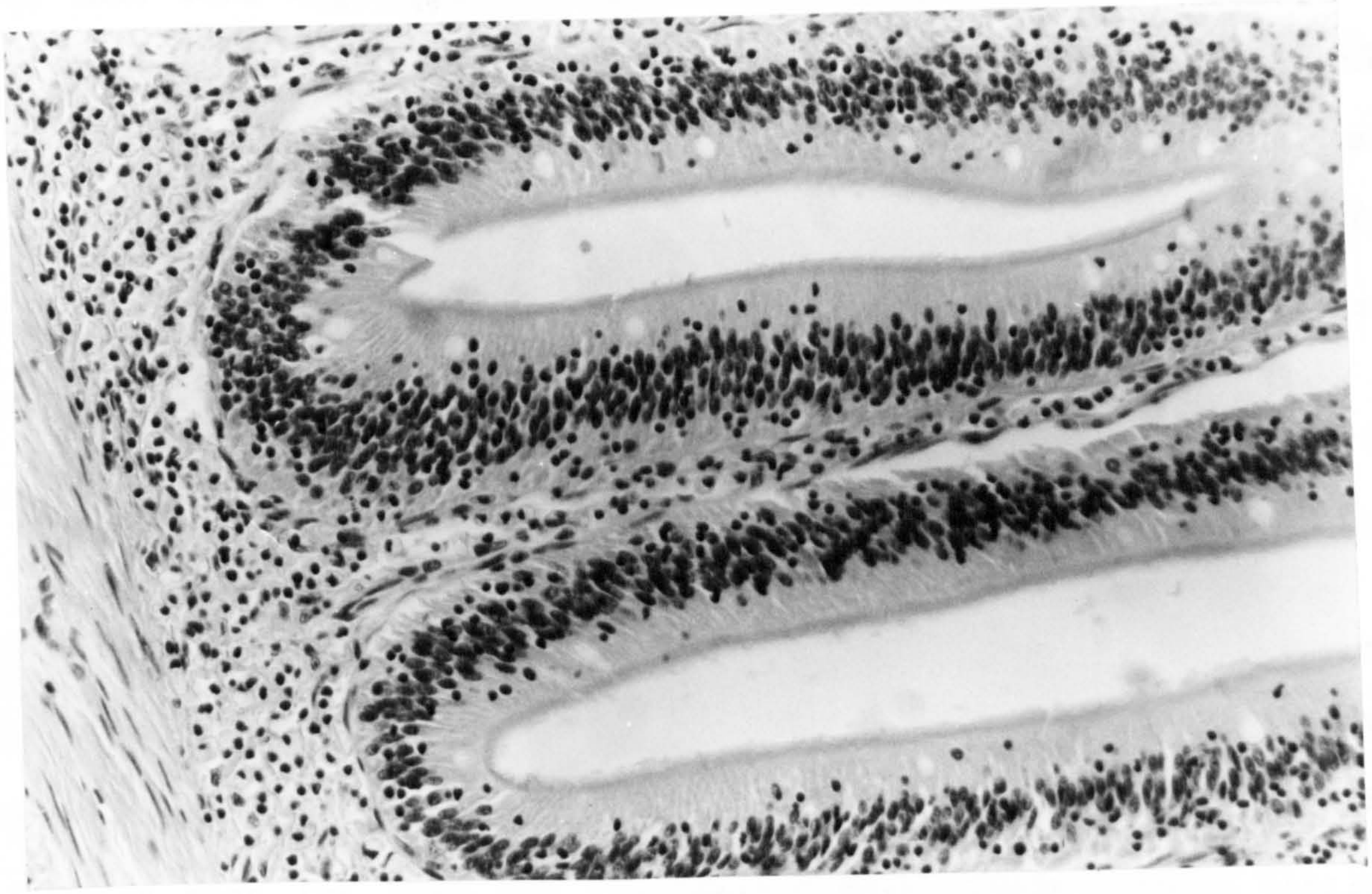


Plate 7.1.b

pathogens or protozoon parasites. Histological examination revealed that the intestinal epithelial cells of fish fed diets containing high phytate, calcium and magnesium levels were characterized by hypertrophy and marked vacuolization of the cytoplasm (Plate 7.1a). In contrast those of fish fed the Control and low phytate diets were slender, columnar-shaped cells with fine granular cytoplasm (Plate 7.1b). Kidney, gills and liver structure in representative fish from all treatment groups appeared to be normal.

7.4 DISCUSSION

From the growth and food utilization data presented in Table 7.7 it should be noted that all of the diets containing phytic acid had weight gains that were significantly ($p < 0.05$) lower than the Control. Fish fed diets containing phytic acid had food conversion ratios (FCRs) significantly ($p < 0.05$) higher than the Control (Table 7.7). Increasing the calcium level in the diet from 0.93% to 1.52% and magnesium from 0.058% to 0.108% did not affect growth. However, increasing the calcium from 0.93% to 2.22% and magnesium from 0.058% to 0.160% slightly but significantly ($p < 0.05$) reduced the weight gain.

Spinelli _et al_. (1983) found that increasing calcium from 0.92% to 1.30% and magensium from 0.054% to 0.085% in trout diets produced comparatively (but not statistically significant) reduced growth rates and feed conversion than for fish fed the Control diet. These authors concluded that increasing calcium level from 0.92% to 1.30% and the magnesium level from 0.054% to 0.085% in trout diet was not sufficient to produce the

effects noted by other investigators (Likuski and Forbes, 1965; Oberleas et al., 1966).

The reduced growth rates of fish on phytic acid diets were influenced by the presence of high dietary calcium and magnesium levels (Diets 6 and 9). This again demonstrates the growth depressing effect of high dietary levels of calcium (and magnesium) and corroborates the findings of Spinelli et al. (1983) in trout and Richardson et al. (1985) in chinook salmon.

Similar growth-depressing effects of excess calcium in phytate containing diets were observed in rats (Likuski and Forbes, 1965; Oberleas et al., 1966; Morris and Ellis, 1980) and in swine (Oberleas et al., 1962). However, reduced growth rates with high phytate diets were presumably due to a progressive decline in protein efficiency ratios (Table 7.7). Moreover, some of the lowered food and protein utilization in phytic acid diets probably stemmed from reduced zinc availability (Tables 7.8, 7.9, 7.10 and 7.12).

Similarly reduced growth and zinc availability were noted by Richardson et al. (1985) in chinook salmon. They found that supplementation of high phytate diets with zinc did partially offset the diminished food and protein conversion, especially when the dietary calcium content was high.

Diets containing phytic acid had lower apparent protein digestibilities than the Control diet (Table 7.7). For example, Diet 9 (1% phytic acid + higher levels of Ca and Mg) had a protein digestibility of 87.12%

compared to 93.14% in the Control. Spinelli et al. (1983) reported that in vitro phytic acid/protein complexes are only partially hydrolysed by pepsin. In vivo tests with rainbow trout in which a casein/phytate complex was substituted for casein showed a 6.6% reduction in protein digestibility. Thus, the lower growth rate and food utilization in phytic acid diets may be attributed to:

(1) the reduced bioavailability of minerals (Likuski and Forbes, 1965; Erdman, 1979; Hartman, 1979);

(2) the impaired food and/or protein digestibility because of formation of phytic acid/protein complexes (Cheryan, 1980; Spinelli et al., 1983).

(3) the depressed absorption of nutrients in the intestine (Plate 7.1a, Richardson et al., 1985).

The final carcass proximate composition of the fish was markedly influenced by the various dietary treatments (Table 7.11). For example, fish fed diets (7, 8, 9 and 11) containing higher levels of phytic acid, calcium and magnesium had higher carcass moisture and lower lipid contents than noted in fish fed the other diets. Moreover, fish fed diets with high calcium and magnesium contents generally had lower levels of protein and lipid than observed in counterparts fed diets with low levels of calcium and magnesium.

Richardson et al. (1985) reported similar changes in the final carcass lipid contents of chinook salmon fed diets containing higher levels of

phytic acid (2.58%) and calcium (5.1%). However, most of the aforementioned changes in body composition are probably ascribable to the depressed food and protein conversion of the various groups.

The abnormal changes found in the intestine of fish fed Diet 9 containing phytic acid, calcium and magnesium content may have resulted from a toxic effect of phytic acid on the epithelial layer of the intestine or impaired magnesium bioavailability due to complexation of magnesium with phytic acid (Erdman, 1979).

Dietary treatments significantly influenced the plasma levels of calcium, zinc and iron but not the levels of magnesium, sodium, potassium and copper (Table 7.8). Dietary calcium and magnesium had no influence on plasma zinc and calcium concentration except when the diets simultaneously contained a high level of phytic acid, calcium and magnesium. Plasma zinc levels were inversely related to dietary phytic acid. Zinc levels in livers, kidney and final carcass were also significantly influenced by the high dietary levels of phytic acid, calcium and magnesium (Tables 7.9, 7.10 and 7.12). These results demonstrate that 1.0% dietary phytic acid reduced zinc bioavailability in carp and this effect was enhanced by the higher levels of calcium (2.22%) and magnesium (0.16%).

Richardson et al. (1985) found reduced bioavailability of zinc in chinook salmon fed diets containing high levels of phytic acid (2.58%) and calcium (5.1%). On the other hand, Spinelli et al. (1983) reported that phytate (at the 0.5% level) did not apparently reduce the bioavailability of zinc in rainbow trout and increasing dietary levels of calcium (from

0.92% to 1.30%) and magnesium (from 0.054% to 0.085%) in the presence of 0.5% phytic acid did not proportionately influence growth or apparent bioavailability of zinc.

The differences in the effect of phytic acid on zinc bioavailability in rainbow trout observed by Spinelli et al. (1983) from the present study and that of Richardson et al. (1985) may be due to species specific responses. Also it may be that the phytic acid level (0.5%) and increasing calcium (1.3%) and magnesium (0.085%) levels were not significant to produce adverse effects.

Dietary phytic acid has been reported to reduce the bioavailability of zinc in rats (Davies and Nitingale, 1975) and swine (Oberleas et al., 1962). Davies and Nitingale (1975) who performed carcass analyses in rats concluded that the addition of 1% phytic acid to diets impaired not only the absorption of dietary zinc, but also the reabsorption of endogenously secreted zinc. The possible mechanism involved in the Zn-Ca-phytate complex formation which is responsible for the reduced bioavailability of zinc has been explained by Byrd and Matrone (1965) and has already been discussed in detail in Section 7.1.

Calcium and magnesium levels in liver, kidney and final carcass were also influenced by dietary phytic acid (Tables 7.9, 7.10 and 7.12). Oberleas (1973) reported that dietary phytate reduced the availability of zinc, iron, calcium and magnesium to monogastric animals by a mechanism involving precipitation of metal-phytate complexes within the intestinal lumen. However, it is interesting to note that increasing the dietary

calcium (2.22%) and magnesium (0.16%) did not improve, rather it depressed, the bioavailability of calcium and magnesium, a trend indicating that it may be as important to Control these elements in the diets as to avoid phytates (Spinelli et al., 1983).

Sodium and potassium levels did not significantly vary in plasma or other organs regardless of the diets fed. Iron content in plasma and kidney showed a slight (but statistically significant) variation with respect to diets (Tables 7.8, 7.10). However, there was no statistical correlation of these values to the dietary phytic acid levels, indicating that phytates do not influence iron bioavailability.

Spinelli et al. (1983) also found a wide range of values for iron levels in blood, kidney and liver in rainbow trout but there was no statistical correlation of these values to the diets and concluded that phytates do not influence iron bioavailability. Similarly Richardson et al. (1985) also observed no significant variation in plasma iron level in chinook salmon fed phytic acid diets.

Dietary treatments influenced copper levels in kidney and liver but not in plasma carcass (Tables 7.8, 7.9, 7.10 and 7.12). Richardson et al. (1985) also found no significant variation in plasma copper levels in chinook salmon fed diets containing phytic acid. In the present study, however, both liver and kidney copper levels decreased as the diets simultaneously contained phytic acid and higher levels of calcium (2.22%) and magnesium (0.16%). Spinelli et al. (1983) reported that liver copper content in trout decreased as the dietary calcium (1.30%) and magnesium

(0.085%) levels increased and suggested that calcium and magnesium play a significant role with respect to regulating copper levels in the blood and liver.

The results of the present study demonstrated that both synthetic and naturally occurring phytic acids were equally effective and resulted in the reduced growth and food utilization in carp. Dietary phytic acid also reduced the bioavailability of some of the minerals, especially calcium and zinc, as reflected by these elements in the plasma, liver, kidney and carcass. Increasing the dietary calcium and magnesium level in the presence of phytic acid significantly influenced growth and mineral (especially Ca, Mg, Zn, Fe, Cu) bioavailability. This reduced growth and mineral bioavailability may be attributable to the formation of insoluble protein-phytate complex and/or phytate-mineral complex.

EXPERIMENT 5.2

Effect of dietary glucosinolate (allyl isothiocyanate) and mustard oilcake on growth and feed utilization in common carp (C. carpio L)

7.5 INTRODUCTION

Brassica seeds and other cruciferae, have long been known to contain thioglucosides commonly known as glucosinolates. The presence of high levels of glucosinolates in Indian Brassica seed meals (mustard and rapeseed) make them undesirable for animal consumption. In particular large amounts of allyl and butenyl isothiocyanates are found to be the main hydrolytic products of these meals (Lo and Bell, 1972). Varying manifestations of toxicity, from depressed weight gain, enlarged thyroids and kidneys to death are observed in rats and other species ingesting glucosinolate hydrolytic products (Srivastava et al., 1975).

Billie et al. (1983) reported that glucosinolate concentrations in diets corresponding to the level in double low, rape varieties (0.2-1.0 mg/g), when fed as the sole protein source, had only minor or no observable physiological consequences on growing rats within the investigated experimental period. However, the results revealed that the concentration (5 mg/g DM) of intact glucosinolates or glucosinolates + myrosinase could cause palatability problems, reduced protein utilization and affect the size of the thyroid, kidney and liver.

Lo and Hill (1971) reported that rats fed diets containing 35% rapeseed meal of Bronowski variety (0.3% glucosinolates) produced comparable avaerage weight gains and feed intakes to those fed a casein based Control diet. On the other hand, Josefsson and Munck (1972) found that rats fed Bronowski meal (0.03% isothiocyanate and 0.19% oxazolidinethione) produced half the weight gain of rats fed a barley meal based Control diet.

In a further study Josefsson and Munck (1973) found that a glucosinolate content corresponding to a level more than 1mg potential isothiocyanate and oxazolidinethione per g of diet depressed the growth of mice as well as feed consumption and protein efficiency ratios. Similarly, Jones (1979) has shown that rats fed on diets with rapeseed meal containing 5-vinyl oxazolidinethione and isothiocyanate at 0.26 mg/g and 0.22 mg/g of diet respectively, depressed the growth to nearly half that of the Control (casein) diet.

Drouliscos and Bowland (1969) reported that rats fed on diets with rapeseed meal containing 0.102% isothiocyanate produced lower growth, net protein utilization and true nitrogen digestibility compared to those fed soybean and casein diets. The thyroid weights in rats fed rapeseed meal were higher than for those fed soybean and casein diets. Clandinin and Bayly (1960) reported that the increase in thyroid size resulting from feeding rapeseed meal to growing chickens was found to be due to an increase in the number of follicles and an increase in the number and size of the epithelial cells in the gland.

Growth depressing and toxic effects of glucosinolates in rapeseed meal have also been reported in fish, particularly in trout and salmonids. Yurkowski et al. (1978) found a weight gain of 28.12g with rainbow trout fed a fish meal and soybean based Control diet. The weight gains were 17.24g, 15.57g and 11.62g when the diet was supplemented with rapeseed protein concentrate, rapeseed meal and rapeseed flour respectively. Total glucosinolate levels in these diets amounted to 0.257 mg/g, 0.730

mg/g and 0.654 mg/g respectively. Hardy and Sullivan (1983) reported that weight gain of rainbow trout was not affected by diets containing up to 20% canola meal (0.23-0.34 mg/g of total glucosinolate). On the other hand Hilton and Slinger (1986), in a study with canola meal in practical-type diets for rainbow trout, concluded that the tolerance level of dietary glucosinolates in young rainbow trout is less than 0.158 mg/g.

Higgs et al. (1982) reported that canola meal between 30% and 32% of dry matter (25% of dietary protein) would not be expected to curtail chinook salmon performance provided that the total glucosinolate content of the diets on a dry basis is less than 0.3 mg/g. In comparison to rainbow trout, in which extreme thyroid hyperplasia was produced after feeding with rapeseed meal containing diets (Yurkowski et al., 1978), common carp appeared to be more resistant to goitrogenic substances in rapeseed (Dabrowski et al., 1982).

The effects of feeding mustard oilcake to fish have not been extensively researched and few reports are available (Chakrabarty et al., 1973; Capper et al., 1982). None of these studies investigated the toxic effects of glucosinolates (in mustard oilcake) on growth and histopathology of carp. Therefore, the present study investigated the toxic effects of different levels of synthetic and natural glucosinolate (i.e. allyl isothiocyanate) on the growth, food utilization and histopathology of carp.

7.6 MATERIALS AND METHODS

7.6.1. Experimental System and Animals

The experimental systems used in the present study are described in detail in Sections 2.1.1 and 2.1.2. The sources of experimental animals and their quarantine procedures are described in Section 2.2.1.

7.6.2. Analytical Techniques

The analytical techniques used were as described in Chapter 2.

7.6.3. Diet Formulation and Preparation

Nine semi-purified isonitrogenous diets were formulated using fish meal and mustard oilcake (containing 4.2 mg/g of allyl isothiocyanate) as protein sources. The specifications of the diets are shown in Table 7.13. The origin and proximate composition of mustard oilcake was the same as for Experiment 4.1. The fish meal used was of UK origin and contained about 77.06% protein, 10.20% lipid, and 12.15% ash on dry weight basis. Formulation of the experimental diets are shown in Table 7.14.

Diets 2, 3, 4 and 5 were formulated to contain 0.5 mg/g, 1.0 mg/g, 2.0 mg/g and 3.0 mg/g of allyl isothiocyanate by replacement of equal amounts of α-cellulose from the diets. Synthetic allyl isothiocyanate ($H_2C = CHCH_2NCS$) was obtained from Aldrich Chemical Co Ltd, Gillingham, Dorset, England. Diets 6 through 9 were formulated by partially substituting mustard oilcake for fish meal to obtain levels of 0.5 mg/g, 1.0 mg/g, 2.0 mg/g and 3.0 mg/g of allyl isothiocyanate from mustard oilcake in Diets

TABLE 7.13

Specification of the experimental diets

DIET NO.
1. Control (Fish meal)
2. Control + 0.5 mg/g allyl isothiocyanate
3. Control + 1.0 mg/g allyl isothiocyanate
4. Control + 2.0 mg/g allyl isothiocyanate
5. Control + 3.0 mg/g allyl isothiocyanate
6. Fish meal + Mustard oilcake contributing 0.5 mg/g allyl isothiocyanate
7. Fish meal + Mustard oilcake contributing 1.0 mg/g allyl isothiocyanate
8. Fish meal + Mustard oilcake containing 2.0 mg/g allyl isothiocyanate
9. Fish meal + Mustard oilcake containing 3.0 mg/g allyl isothiocyanate

TABLE 7.14

Formulations of the diets used in Experiment 5.2

	DIET NO.								
	1	2	3	4	5	6	7	8	9
Fish meal	51.90	51.90	51.90	51.90	51.90	46.19	40.48	29.06	16.83
Mustard oilcake	-	-	-	-	-	11.90	23.81	47.62	71.42
Cod liver oil	-	-	-	-	-	0.29	0.87	2.04	3.25
Soybean oil	4.70	4.70	4.70	4.70	4.70	4.06	3.11	1.23	-
Mineral mixture[1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin premixes[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Binder (CMC)[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Dextrin	25.00	25.00	25.00	25.00	25.00	22.06	17.23	9.55	-
α-cellulose	9.90	9.85	9.80	9.70	9.60	7.00	6.00	2.00	-
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Allyl isothiocyanate	-	0.05	0.10	0.20	0.30	-	-	-	-
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1] For the composition of the mineral and vitamin premixes see Tables 2.3 and 2.4 respectively

[2] Carboxymethyl cellulose (high viscosity)

TABLE 7.15

Analysed composition of the diets used in Experiment 5.2

(% dry matter basis)

Components	DIET NO.								
	1	2	3	4	5	6	7	8	9
Dry matter	93.95	94.48	94.51	94.93	95.11	93.90	94.25	94.38	93.54
Crude protein	41.04	40.73	40.97	40.64	40.91	40.67	40.39	40.07	39.80
Crude lipid	9.82	9.90	10.10	9.78	9.72	9.70	9.94	10.08	10.20
Ash	10.12	10.15	10.13	10.16	10.18	10.82	11.35	12.57	13.24
Crude fibre	7.05	7.08	7.04	7.10	7.12	6.95	7.57	7.08	8.89
NFE[1]	31.97	32.14	31.76	32.32	32.07	31.86	30.75	30.20	27.87
Chromic oxide	0.49	0.50	0.49	0.48	0.48	0.49	0.50	0.51	0.49
Allyl isothiocyanate	-	0.035	0.072	0.14	0.23	0.04	0.09	0.18	0.28

[1]Nitrogen free extractive calculated as

100 - (Moisture+Crude protein+Crude lipid+Ash+Crude fibre)

TABLE 7.16

Analysed amino acid composition of the diets used in Experiment 5.2

(% dry matter basis)

Amino acids	DIET NO.									Requirement for carp[1]
	1	2	3	4	5	6	7	8	9	
Arginine	2.60	2.58	2.57	2.62	2.62	2.58	2.62	2.47	2.34	1.52
Histidine	0.75	0.76	0.75	0.74	0.74	0.75	0.76	0.86	0.89	0.56
Isoleucine	1.62	1.64	1.61	1.65	1.64	1.62	1.58	1.55	1.50	0.92
Leucine	2.59	2.58	2.60	2.63	2.66	2.66	2.58	2.55	2.58	1.64
Lysine	2.81	2.84	2.86	2.85	2.80	2.82	2.68	2.30	2.01	2.12
Methionine	1.03	1.00	0.99	1.06	1.04	1.00	0.96	0.84	0.56	0.64
Cystine	0.46	0.44	0.45	0.46	0.45	0.44	0.44	0.38	0.36	-
Phenylalanine	1.58	1.56	1.55	1.59	1.60	1.58	1.54	1.44	1.48	1.16
Tyrosine	1.08	1.10	1.09	1.07	1.08	1.06	1.04	1.00	0.88	-
Threonine	1.69	1.71	1.66	1.65	1.69	1.68	1.64	1.64	1.66	1.32
Valine	2.14	2.18	2.12	2.24	2.16	2.12	2.10	2.06	1.82	1.16
Alanine	2.42	2.38	2.35	2.34	2.36	2.34	2.22	2.14	1.60	-
Aspartic acid	3.32	3.25	3.22	3.38	3.30	3.28	3.24	3.36	4.00	-
Glutamic acid	5.18	5.04	5.11	5.24	5.12	5.10	5.16	5.26	5.87	-
Glycine	2.20	2.22	2.18	2.19	2.22	2.18	2.16	2.14	1.89	-
Proline	1.78	1.81	1.75	1.77	1.78	1.76	1.75	1.86	2.00	-
Serine	1.38	1.41	1.44	1.40	1.39	1.40	1.42	1.38	1.35	-

[1] Data for EAA requirement of carp from Ogino (1980)

TABLE 7.17

A summary of the methodology used to evaluate the effects of glucosinolate (allyl isothiocyanate) on growth and food utilization of common carp (C. carpio)

Fish	C. carpio mean initial weight 4.17±0.013g
Duration of Experiment	Eight weeks
Treatment	Evaluation of the toxic effects of glucosinolate (allyl isothiocyanate) on the growth and food utilization in common carp
No. of Treatment	Nine
Replication	3/treatment
Water Temperature	27 ± 1°C
Stocking Density	12 fish/tank
Feeding Rate	6% of body weight, three times per day
Carcass Sampling	Initial sample - 15 fish at the start of the experiment Final sample - 12 fish per treatment (4 fish from each replicate)
Faeces Collection	Collected twice daily - morning and evening - for two weeks, dried in an oven at 60°C and kept in air-tight containers until used for subsequent chemical analysis for digestibility study
Physicochemical characteristics of water monitored	Temperature, pH, dissolved oxygen and total ammonia

6, 7, 8 and 9 respectively.

All the diets were formulated to contain 0.5% chromic oxide to study nutrient digestibility. Carboxymethyl cellulose (CMC) and dextrin were used as binder and carbohydrate sources respectively. The composition of mineral and vitamin premixes used are shown in Section 2.4. Diets were prepared using a California pellet mill (Model CL2) using a 3mm die. The mixing of ingredients was as in other experiments.

All the diets were analysed for proximate and amino acid composition and the results are presented in Tables 7.15 and 7.16 respectively. The allyl isothiocyanate contents of the diets were analysed by the methods described in Section 2.7.1.

7.6.4. General Experimental Procedure

Each treatment had three replicates, 12 fish per replicate, with mean initial weight of 4.17±0.013g. Acclimation and weighing procedures were as described earlier (Section 2.2.3). During the acclimation period fish were fed trout pellet (Ewos Baker's Omega No.3, 49% protein). Fish were bulk weighed weekly, after 12 hours starvation, during the experimental period. The experiment was conducted for a period of eight weeks. A summary of the methodology used in the present study is given in Table 7.17.

7.6.5. Feeding Rates

The fish were fed three times daily between 09.00 and 17.00 hours at four

hourly intervals. Fish were fed at 6% of their body weight per day. As the moisture contents of the diets were low and similar (Table 7.15) no correction of feeding rate was made for moisture content. Details of food administration are given in Section 3.2.4. A record of the amount of food fed was kept for the subsequent calculation of food conversion and protein utilization. The quantity of food fed per day was adjusted after each weekly weighing and fed for the subsequent week.

7.6.6. Faeces Collection

Fish were transferred to the faecal collection system (Section 2.1.2) at the beginning of the seventh week and faeces were collected twice daily - morning and evening - for two weeks. Details of faecal collection procedure are described in Section 3.2.5.

7.6.7. Histological Techniques

Histological techniques were as described in Section 3.2.7. Fifteen fish per treatment were sampled for histopathology to assess changes in gills, thyroid, liver, muscle, kidney and intestine.

7.6.8. Analysis of Experimental Data

Experimental results were analysed as described in Section 2.8.1. Statistical analyses were performed as described in Section 2.9.1.

7.7 RESULTS

7.7.1. Analysed Composition of the Diets

Proximate analyses of the experimental diets are shown in Table 7.15. Moisture contents varied from 4.82% to 6.46% and protein from 39.80% to 41.04%. Lipid, crude fibre and ash contents showed little variation between diets. Allyl isothiocyanate levels were markedly different from those formulated showing a loss of as high as about 30% of the supplemental level (Table 7.15). This could possibly be because of the heat involved during pelleting and the volatile nature of the ally isothiocyanate.

7.7.2. Growth

Growth performances of carp fed the experimental diets are presented as mean initial and final weight, weight gain, percentage weight gain and specific growth rate in Table 7.18 and graphically in Figure 7.2. Mean initial weights were insignificantly ($p > 0.05$) different. There were no significant ($p > 0.05$) differences between the weight gains of fish fed the Control (Diet 1) and Diets 2 and 6 (0.35 mg/g and 0.40 mg/g of allyl isothiocyanate respectively) but these values were significantly ($p < 0.05$) higher than those for the rest of the diets. However, there was no significant ($p > 0.05$) difference between the weight gains of fish fed the Diets 4 and 7.

Specific growth rates (SGRs) for the various dietary treatment groups ranged from 2.50 to 3.51. There were no significant differences ($p > 0.05$) between the SGRs of the Control (Diet 1) and Diets 2 and 6 but

TABLE 7.18

Growth and food utilization of common carp fed the experimental diet for eight weeks

	DIET NO.									± S.E.[2]
	1	2	3	4	5	6	7	8	9	
Initial weight (g)	4.16[a1]	4.15[a]	4.18[a]	4.19[a]	4.18[a]	4.16[a]	4.16[a]	4.18[a]	4.16[a]	0.01
Final weight (g)	29.69[a]	29.36[a]	27.08[b]	25.08[c]	23.00[d]	29.27[a]	25.66[c]	21.58[e]	16.93[f]	0.37
Weight gain (g)	25.53[a]	25.21[a]	22.90[b]	20.89[c]	18.81[d]	25.11[a]	21.50[c]	17.40[e]	12.77[f]	0.35
% weight gain	613[a]	608[a]	548[b]	498[c]	450[d]	603[a]	517[c]	417[e]	307[f]	6.60
SGR (% day)	3.51[a]	3.49[a]	3.34[b]	3.19[c]	3.05[d]	3.48[a]	3.25[c]	2.93[e]	2.50[f]	0.02
SGR as % of Control	100	99.4	95.2	90.8	86.9	99.1	92.6	83.5	71.2	-
FCR	1.41[a]	1.44[ab]	1.49[bc]	1.61[d]	1.69[e]	1.43[a]	1.53[c]	1.72[e]	2.06[f]	0.02
PER	1.72[a]	1.69[a]	1.64[b]	1.53[c]	1.44[d]	1.72[a]	1.62[b]	1.45[d]	1.22[e]	0.014
ANPU (%)	30.85[a]	30.04[abc]	28.90[bcd]	27.66[d]	23.90[e]	30.66[ab]	28.49[cd]	25.20[e]	21.06[f]	0.52

[1] Figures in the same row having the same superscripts are not significantly ($p > 0.05$) different

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

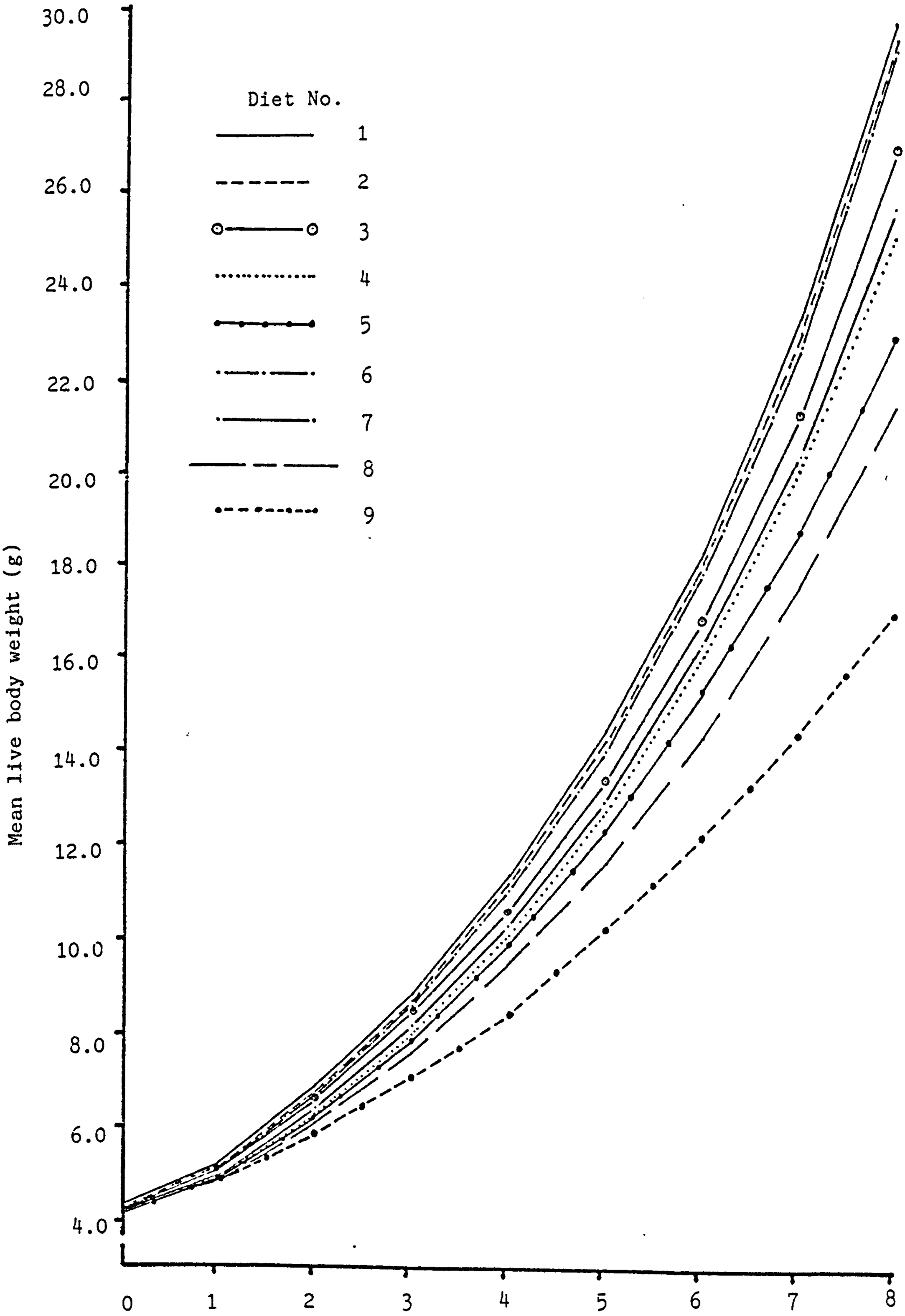


FIGURE 7.2 Growth responses of fish fed experimental diets for 8 weeks

these values were significantly higher than for the rest of the diets. Again, there was no significant difference ($p > 0.05$) between the SGRs of Diets 4 and 7 but these values were significantly ($p < 0.05$) lower than for Diet 3.

7.7.3. Food Conversion Ratio (FCR)

Mean food conversion ratios (FCRs) obtained for each experimental diet are presented in Table 7.18. FCRs were relatively low and Diets 1 (Control), 2 and 6 produced significantly lower FCRs. There were no significant ($p > 0.05$) differences between the FCRs of Diets 2 and 3 and 3 and 7 respectively. These FCRs were significantly ($p > 0.05$) higher than for Diets 4, 5, 8 and 9.

7.7.4. Protein Utilization

The efficiency with which fish utilized dietary protein was determined by calculation of protein efficiency ratio (PER) and apparent net protein utilization (ANPU) (Table 7.18). PERs followed similar trends to FCRs with Diets 1, 2 and 6 producing significantly ($p < 0.05$) the highest PERs. However, there was no significant ($p > 0.05$) difference in PER between Diets 3 and 7 but these values were significantly ($p < 0.05$) higher than for Diets 4, 5, 8 and 9.

Mean apparent net protein utilization (ANPU %) was calculated for all dietary treatments and is presented in Table 7.18. The ANPU values were relatively high ranging from 21.06 to 30.85. There were no significant differences between ANPUs of Diets 1, 2 and 6; 2, 3 and 6; 2, 3 and 7

and 3, 4 and 7 respectively.

7.7.5. Apparent Nutrient Digestibility

Apparent nutrient digestibilities of the experimental diets were determined as earlier (Section 2.8.1) and the results are presented in Table 7.19. Apparent dry matter digestibility ranged from 58.42% to 68.27%. Apparent protein digestibility values for all diets were fairly high and ranged from 80.44% to 87.40% with the Control diet having the highest value. Apparent lipid and ash digestibility values ranged from 87.18% to 92.82% and 33.19% to 42.48% respectively.

7.7.6. Carcass Composition

Proximate carcass composition data for fish at the start and at the end of the experiment are presented in Table 7.20. Fish from Diet 9 had significantly ($p < 0.05$) the highest (79.14%) moisture and lowest lipid content (4.22%). There were no significant ($p > 0.05$) differences between carcass moisture contents of fish fed Diets 1 (Control), 2, 3 and 6. In general, fish fed diets with higher levels of allyl isothiocyanate had higher moisture contents and lower lipid contents.

The final carcass protein levels ranged from 14.08% to 14.66%. There were no significant ($p > 0.05$) differences between carcass protein contents of fish fed Diets 1, 2, 3, 6 and 7 but these values were significantly ($p < 0.05$) higher than for the rest of the diets.

There were no significant differences between the carcass protein con-

TABLE 7.19

Apparent nutrient digestibility (%) of the experimental diets

	DIET NO.								
	1	2	3	4	5	6	7	8	9
Dry matter digestibility	68.27	66.59	64.29	63.52	60.28	66.06	64.88	61.12	58.42
Crude protein digestibility	87.40	87.24	85.81	84.20	82.46	87.18	86.26	83.16	80.44
Crude lipid digestibility	92.80	92.27	91.82	90.10	88.64	92.36	91.45	89.16	87.18
Ash digestibility	42.48	40.35	39.81	38.17	35.26	40.62	38.61	36.02	33.19

*No statistical analysis was possible as determinations were performed on pooled samples

TABLE 7.20

Proximate carcass composition (% fresh matter basis) of fish samples at the start and the end of the experiment

	Initial (all fish)	FINAL Diet No.									± S.E.[2]
		1	2	3	4	5	6	7	8	9	
Moisture	81.10	77.17^{a1}	77.25^{ab}	77.32^{ab}	77.66^{c}	78.14^{d}	77.23^{a}	77.65^{bc}	78.03^{cd}	79.14^{e}	0.13
Crude protein	13.08	14.66^{a}	14.61^{a}	14.43^{ab}	14.27^{bc}	14.21^{bc}	14.62^{a}	14.42^{ab}	14.22^{bc}	14.08^{c}	0.08
Crude lipid	3.22	5.49^{a}	5.45^{ab}	5.27^{bc}	5.09^{cd}	4.95^{de}	5.42^{ab}	5.06^{d}	4.80^{e}	4.22^{f}	0.06
Ash	2.42^{a}	2.53^{a}	2.49^{a}	2.64^{a}	2.68^{a}	2.55^{a}	2.53^{a}	2.57^{a}	2.50^{a}	2.43^{a}	0.06
TOTAL	99.82	99.85	99.80	99.66	99.70	99.85	99.80	99.70	99.55	99.87	-

[1] Figures in the same row having the same superscripts are not significantly ($p > 0.05$) different

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

tents of fish fed Diets 3, 4, 5, 7 and 8 and 4, 5, 8 and 9 respectively. Carcass lipid contents ranged from 4.22% to 5.49% with fish fed diets 1, 2 and 6 producing significantly ($p < 0.05$) the highest levels. There were no significant differences in carcass ash content.

7.7.7. General Health and Histological Examination

At the end of the experiment no abnormalities or physical deformities were observed in fish examined from the various dietary treatment groups. Histopathological examination of the gills, liver, muscle, intestine, thyroid and kidney revealed that fish fed diets (4, 5, 8 and 9) containing higher levels of glucosinolate showed histologically active thyroid. Other organs in representative fish in all treatment groups were judged to be normal.

As discussed earlier (Section 3.3.4), thyroid tissue in carp is usually distributed being located in pharyngeal and renal tissues. Thyroid tissues in the present study in all groups were distributed in pharyngeal and kidney tissues.

Fish fed the Control diet had normal thyroid and had fewer follicles. These follicles were circular in shape with abundant pink colloid with a single-cell-thick layer of squamous epithelium. Fish fed Diets 4, 5, 8 and 9 showed an increase in follicle number in the kidney tissues (Plate 7.2). Follicles displayed a wide range of sizes and shapes. Some follicles had little colloid. The epithelial cells were flat and cell heights were slightly greater than that in the Control.

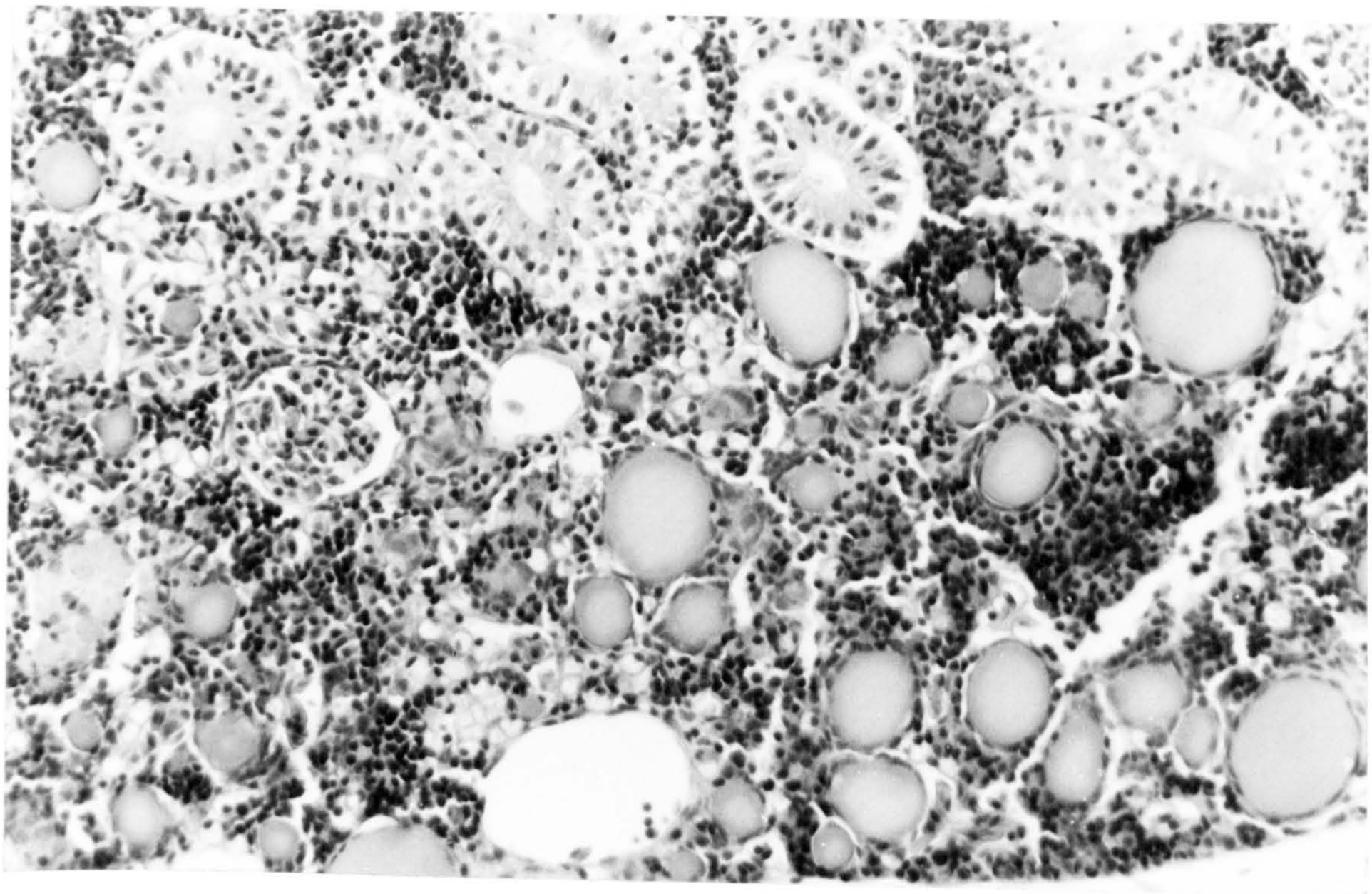


PLATE 7.2

Thyroid follicles in kidney tissues of carp fed diets (4, 5, 8 and 9) containing higher levels of allyl isothiocyanate from Experiment 5.2. Note variable sizes and general packed appearance of follicles. Some follicles had little colloid.

(x250)

PLATE 7.3.a

Thyroid follicles in pharyngeal region of carp fed fish meal based Control diet from Experiment 5.2. Note no visible epithelial hyperplasia and circular shape of the follicles.

(x250)

PLATE 7.3.b

Thyroid follicles in pharyngeal region of carp fed diets (4, 5, 8 and 9) containing higher levels of allyl isothiocyanate from Experiment 5.2. Note marked epithelial hyperplasia.

(x250)

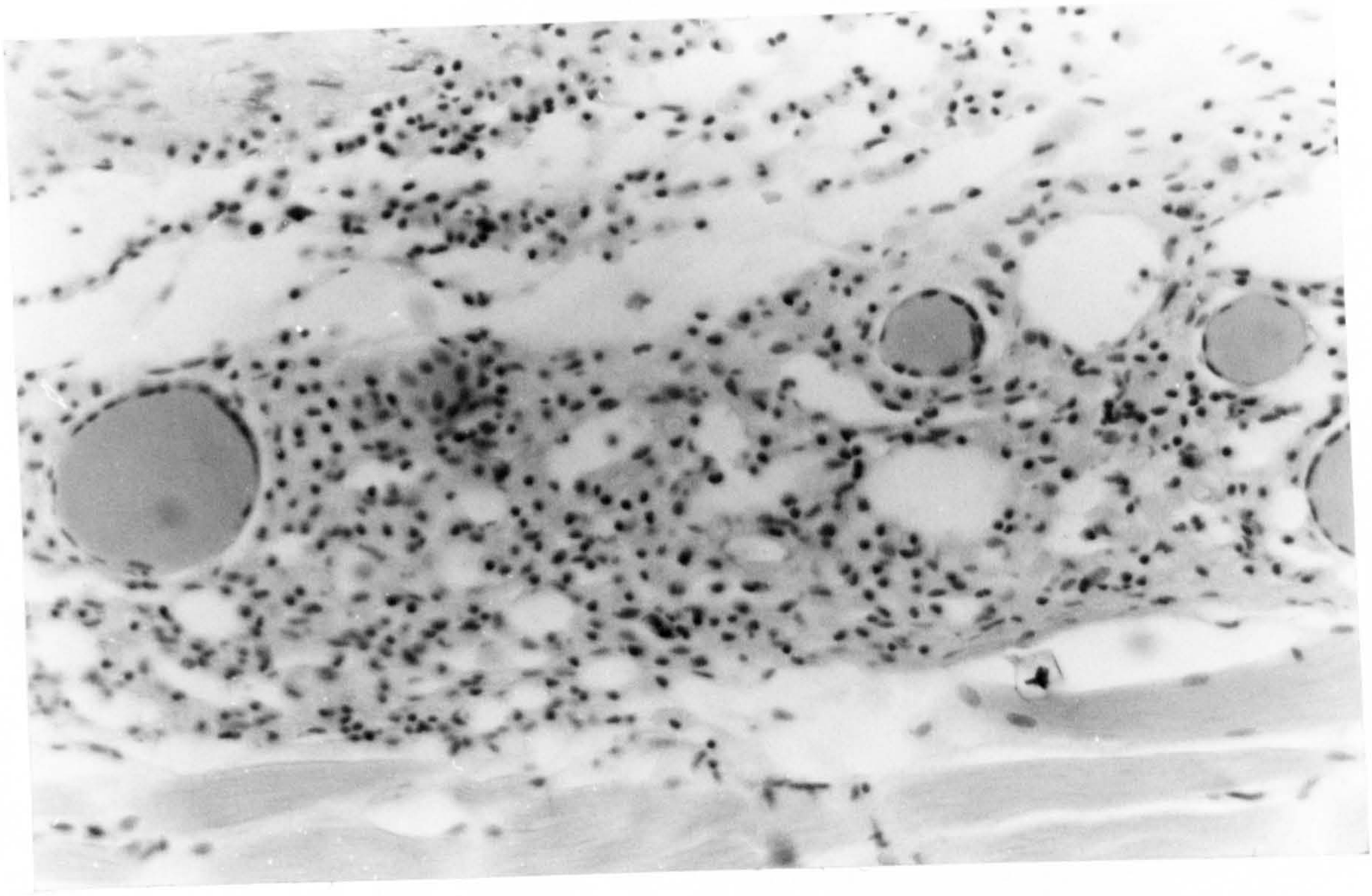


Plate 7.3.a

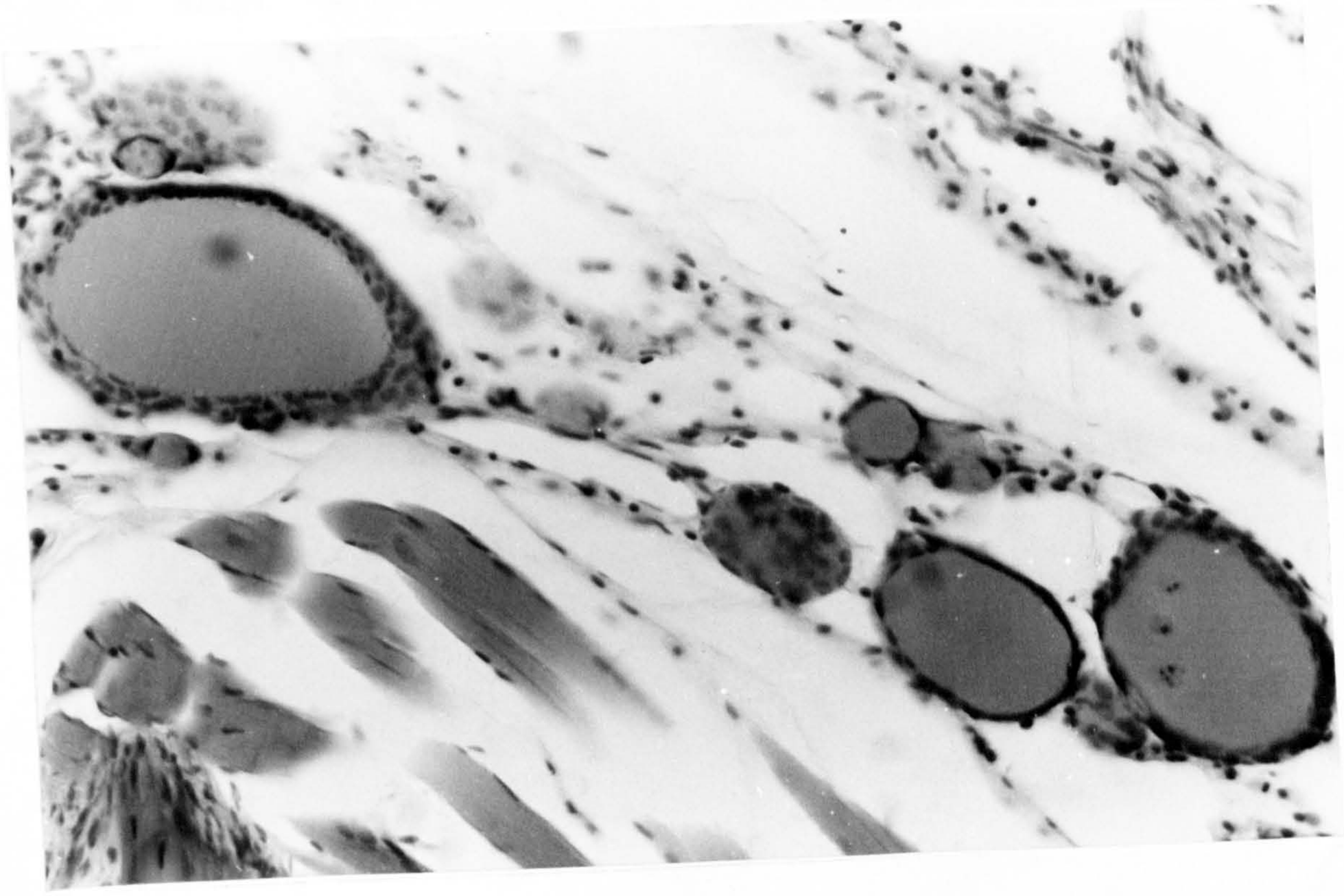


Plate 7.3.b

As for kidney, follicles in the pharyngeal region also showed an increase in number in fish fed Diets 4, 5, 8 and 9 and displayed a wide range of sizes. Marked epithelial hyperplasia was observed with a double (thick) layer of epithelial cells (Plate 7.3b). Colloid material in these follicles was vacuolated and sometimes depleted within the follicles.

7.8 DISCUSSION

In this study carp fed diet (6) containing about 12% mustard oilcake (0.4 mg/g allyl isothiocyanate) had weight gains statistically similar to those of fish fed the Control diet. Comparison of the growth performance of fish fed diets (2, 3, 4 and 5) containing allyl isothiocyanate and diets (6, 7, 8 and 9) containing mustard oilcake (contributing different levels of allyl isothiocyanate) illustrated that allyl isothiocyanate depresses growth.

The comparatively greater growth depression in fish fed Diets 6, 7, 8 and 9 (containing mustard oilcake) may have been due to the presence of other anti-nutritional factors in mustard oilcake, such as tannins. Moreover, Diet 9 (2.8 mg isothiocyanate/g diet) was also slightly deficient in EAAs such as lysine and methionine.

Fish fed Diets 1, 2 and 6 produced significantly higher SGRs. Although SGRs of fish fed Diets 3 and 7 (containing 0.72mg and 0.9mg allyl isothiocyanate/g diet respectively) were significantly ($p < 0.05$) lower than the Control, they produced about 95% and 93% respectively of the Control diet SGR.

Food conversion ratios in this study are fairly low compared to those found in the previous studies (Sections 3.3 and 6.3). The FCR on Diet 7 (1.53) was lower than the FCR found by Hasan (1986) using the same level of mustard oilcake in a carp fry diet. However, these FCRs were higher than the values, 1.35 and 1.21, reported by Capper et al. (1982) using 20% roasted mustard seed cake in fingerling carp diets.

Dry matter digestibilities varied with the levels of allyl isothiocyanate or mustard oilcake in the diet. Despite this, apparent protein digestibility (APD) values were fairly high. The APD of Diet 7 is comparable to that found by Hasan (1986) using the same level of mustard oilcake in a carp fry diet. However, it may be noted that, in general, digestibility values were slightly higher in diets (2, 3, 4 and 5) containing synthetic allyl isothiocyanate than in those diets (6, 7, 8 and 9) containing mustard oilcake. This is probably due to the slightly higher level of allyl isothiocyanate in these diets as well as to other anti-nutritional factors (e.g. tannins) in diets containing mustard oilcake.

Carcass moisture and lipid contents varied little although fish fed Diet 9 had the lowest lipid and highest moisture content. Higgs et al. (1982) observed a reduction in percent lipid in the body of chinook salmon fed canola meal diets containing various levels of glucosinolates compared to a Control diet. The reduction in carcass lipid and the inverse relationship between moisture and lipid as reported by other workers has been discussed earlier in Section 3.4. Carcass protein levels were little affected by dietary treatments.

Marked thyroid hyperplasia was observed in fish fed the diets (4, 5, 8 and 9) containing higher levels of glucosinolates (allyl isothiocyanate). Abnormalities in the thyroid tissues indicate an inhibition of thyroid hormone production. Influences of rapeseed meal diets on thyroid function have been demonstrated in mammals and birds. Reported effects include thyroid enlargement (Clandinin et al., 1966; Lo and Hill, 1971; Belzile et al., 1974), reduced synthesis of intrathyroidal iodocompounds, reduced levels of plasma protein-bound iodine (PBI) or thyroxine T4 (Lo and Hill, 1971). In most cases, these effects have been attributed to isothiocyanate and goitrin (e.g. venyloxazolidinethione) formed from glucosinolates in the feed by myrosinase (Van Etten et al., 1966).

Allyl isothiocyanate from sinigrin (glucosinolate of mustard oilcake) is easily transformed into thioamides. Thus, the effect of sinigrin on the thyroid gland may be as a result of thioamides formed from the isothiocyanate influencing the entire metabolism, growth and development of the animals (Billie et al., 1983).

Glucosinolate from rapeseed meal has been reported to cause depressed growth, feed intake and feed utilization and histological changes in the liver, kidney and thyroid in coho salmon (Higgs et al., 1979). The studies of Yurkowski et al. (1978) demonstrated that glucosinolate induced histological aberration of the thyroid of trout may be accompanied by reduced levels of plasma T4 and by concomittant depression in growth rate.

Hardy and Sullivan (1983) and Hilton and Slinger (1986) also observed similar histopathological changes in the thyroid of rainbow trout fed canola meal containing various levels of glucosinolates. However, criteria used in the present experiment based on histological observation alone to assess thyroid status in a single sample at the end of the experiment may not provide a valid index of the thyroid activity (Yurkowski et al., 1978). Future studies should include continuous monitoring of plasma T3 and T4 levels as well as histological criteria to assess thyroid status.

The significant reduction in growth and the thyroid histological abnormalities of carp fed diets containing above 0.40 mg/g of allyl isothiocyanate indicate that allyl isothiocyanate level above 0.4 mg/g is toxic to carp. Therefore, it is concluded that the tolerance limit of dietary glucosinolate (allyl isothiocyanate) in carp is less than 0.40 mg/g, the minimum level of glucosinolate supplied by mustard oilcake in this study.

No reports appear in the literature concerning the tolerance limit of allyl isothiocyanate or total glucosinolate from mustard oilcake in fish to evaluate the suitability of mustard oilcake in fish feed. However, the above mentioned level (0.40 mg/g) is higher than the maximum 0.3mg total glucosinolate/g diet suggested by Higgs et al. (1982) for chinook salmon and the 0.23-0.34mg total glucosinolate/g diet used without effect in trout by Hardy and Sullivan (1983). It is possible that carp used in this study are more resistant to dietary glucosinolates than either salmon or trout.

In comparison to rainbow trout, in which extreme thyroid hyperplasia was produced after feeding with rapeseed meal containing diets (Yurkowski et al., 1978), common carp appeared to be more resistant to the goitrogenic substances in rapeseed (Dabrowski et al., 1982).

The toxic effect of glucosinolate (allyl isothiocyanate) used in this study could be different from the glucosinolate (butenyl isothiocyanate in rapeseed) used by Higgs et al. (1982). Isothiocyanates are considered to be fairly toxic compounds when administered subcutaneously (Jenner et al., 1964; Nishie and Daxenbichler, 1980) but according to the high reactivity (Olsen and Sorensen, 1980) of these compounds it is unlikely that they are absorbed as isothiocyanate from the intestinal canal (Langer and Stolc, 1965).

The results of the present study demonstrated that glucosinolate (allyl isothiocyanate) at the level of 0.4 mg/g or mustard oilcake at the level of about 12% can be included in the carp diet without inhibiting the growth performance or causing no adverse changes in histology of various tissues.

CHAPTER 8 : EXPERIMENT 6

Partial substitution of fish meal by oilseed meals in various combination in the diet of common carp (*C. carpio* L)

8.1 INTRODUCTION

Attempts to partially or completely replace the fish meal component of practical fish feed with commercially available plant proteins have met with variable success, and in most cases have resulted in reduced growth and food conversion efficiency at higher dietary inclusion levels (Atack et al., 1979; Dabrowski and Kozak, 1979; Jackson et al., 1982; Viola et al., 1982).

In most cases, single plant protein sources were evaluated at various inclusion levels to substitute fish meal in the diet (Jackson et al., 1982; Hilton and Slinger, 1986). The majority of the plant protein sources tested were oilseed meals. Oilseeds are known to contain a variety of growth inhibiting anti-nutritional factors (Liener, 1975). When a higher level of plant protein is included, the anti-nutritional factors in the diets exceed the tolerance limit of the test animal. This often leads to reduced growth and food utilization.

The use of different plant protein sources in combination can prevent high inclusion levels of any single anti-nutritional component in the diet.

Combinations of different plant protein sources in Diets for O. mossambicus were also advocated by Jackson et al. (1982) as a means of compensating for essential amino acid deficiency in any single protein source.

Several authors have reported comparatively better growth performances of fish fed diets containing combinations of plant protein sources. Olukunle (1982) found improved performance of O. mossambicus fry fed a diet containing groundnut and sunflower seed meal as partial substitutes for fish meal protein compared with diets containing groundnut or sunflower meal alone. Richards (1983) also noted that a combination of sunflower and sesame meals resulted in better growth of O. mossambicus than when the meals were tested singly.

Fukuda and Kesamaru (1972) reported that diets prepared with wheat germ, white fish meal, defatted rice bran and magna yeast showed improved feed efficiency in common carp (C. carpio) compared to diets prepared with white fish meal and wheat germ or commercial carp diets.

In the previous experiments (Chapters 3 and 6) diets containing mustard oilcake, sesame and linseed meals, used as single plant protein sources, resulted in poor growth performance and food utilization. This reduced growth performance was principally ascribed to the higher levels of anti-nutritional factors contributed by the plant protein source. In view of this in the present study an attempt was made to partially replace fish meal, keeping the anti-nutritional factors low, by various combinations of the above mentioned plant protein sources in the diet of carp.

8.2 MATERIALS AND METHODS

8.2.1. Experimental Systems and Animals

The experimental systems used in this study are described earlier (Section 2.1.1 and 2.1.2). The sources of experimental animals and their quarantine procedures are described in Section 2.2.1.

8.2.2. Analytical Techniques

The analytical techniques used were as described in Chapter 2.

8.2.3. Diet Formulation and Preparation

Nine semi-purified isonitrogenous diets were formulated using fish meal, mustard oilcake, linseed and sesame meal as protein sources. The specifications of the experimental diets are shown in Table 8.1. The origin of the linseed and sesame meals were as in Experiment 4.2 (Chapter 6). The mustard oilcake used was a new batch obtained from Bangladesh. The fish meal used was of UK origin. Proximate composition and antinutritional factors in the plant protein sources are shown in Table 8.2. All the diets were formulated to contain 40% protein and the formulations of the experimental diets are shown in Table 8.3. Plant proteins were tested at two different (25% and 40% of total protein) inclusion levels (Table 8.1).

Each diet was formulated to contain 0.5% chromic oxide to study the nutrient digestibility of the experimental diets. Carboxymethyl cellulose and dextrin were used as binder and carbohydrate source

TABLE 8.1

Specification of the experimental diets used in Experiment 6

Diet No.	Percentage of total protein contributed by various sources in the diets			
	Fish meal	Mustard oilcake	Linseed meal	Sesame meal
1 (Control)	100.00	-	-	-
2	75.00	8.33	8.33	8.33
3	75.00	12.50	6.25	6.25
4	75.00	6.25	12.50	6.25
5	75.00	6.25	6.25	12.50
6	60.00	13.33	13.33	13.33
7	60.00	20.00	10.00	10.00
8	60.00	10.00	20.00	10.00
9	60.00	10.00	10.00	20.00

TABLE 8.2

Proximate composition and anti-nutritional factor contents of the ingredients used in Experiment 6 (% dry matter basis)

	Ingredients			
	Fish meal	Mustard oilcake	Linseed meal	Sesame meal
Dry matter	90.39	90.34	89.65	87.60
Crude protein	76.29	34.58	32.96	36.69
Crude lipid	10.19	12.26	2.81	2.48
Ash	13.02	11.36	11.86	13.20
Crude fibre	0.50	12.04	9.08	23.11
NFE[1]	-	29.76	43.29	24.52
Allyl isothiocyanate	-	0.36	-	-
Phytic acid	-	-	2.45	2.38
Hydrocyanic acid	-	-	0.032	-
Tannins	-	1.64	-	-

[1] Nitrogen free extractives calculated as

100 - (Moisture+Crude protein+Crude lipid+Ash+Crude fibre)

TABLE 8.3

Formulation of the diets used in Experiment 6

	DIET NO.								
	1	2	3	4	5	6	7	8	9
Fish meal	52.43	39.32	39.32	39.32	39.32	31.46	31.46	31.46	31.46
Mustard oilcake	-	9.64	14.46	7.23	7.23	15.42	23.13	11.57	11.57
Linseed meal	-	10.11	7.58	15.17	7.58	16.18	12.14	24.27	12.14
Sesame meal	-	9.08	6.81	6.81	13.63	14.54	10.90	10.90	21.80
Cod liver oil	-	1.00	1.00	1.00	1.00	1.80	1.80	1.80	1.80
Soybean oil	4.66	3.30	2.84	3.51	3.56	2.29	1.55	2.62	2.69
Mineral premixes[1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin premixes[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Binder (CMC)[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Dextrin	25.00	15.53	15.75	14.61	16.22	9.81	10.19	8.38	10.96
-cellulose	9.41	3.52	3.74	3.85	2.96	-	0.33	0.50	0.08
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1] For composition of the mineral and vitamin premixes see Tables 2.3 and 2.4 respectively

[2] Sodium carboxymethyl cellulose (high viscosity)

TABLE 8.4

Analysed composition of the diets used in Experiment 6

(% dry matter basis)

	DIET No.								
	1	2	3	4	5	6	7	8	9
Dry matter	95.00	94.27	94.32	94.38	94.84	94.37	94.13	94.48	94.39
Crude protein	40.21	40.51	41.17	41.00	40.66	40.20	39.95	40.12	40.53
Crude lipid	9.82	10.06	9.98	10.05	10.12	9.89	10.10	9.96	9.90
Crude fibre	8.24	7.42	7.95	8.65	7.62	7.17	7.31	7.10	7.52
Ash	10.83	12.78	12.76	12.84	12.88	13.89	13.80	13.76	13.70
NFE[1]	30.90	29.23	28.14	27.46	28.72	28.85	28.84	29.06	28.35
Chromic oxide	0.48	0.49	0.50	0.48	0.50	0.49	0.48	0.49	0.48
Allyl isothiocyanate[2]	-	0.034	0.052	0.026	0.026	0.055	0.083	0.041	0.041
Phytic acid[2]	-	0.46	0.35	0.53	0.51	0.74	0.56	0.85	0.82
Hydrocyanic acid[2]	-	0.003	0.002	0.005	0.002	0.005	0.004	0.008	0.004
Tannins[2]	-	0.16	0.23	0.12	0.12	0.25	0.38	0.19	0.19

[1]Nitrogen free extractives calculated as

100 - (Moisture+Crude protein+Crude lipid+Ash+Crude fibre)

[2]Levels of anti-nutritional factors were estimated from their levels in the dietary protein sources determined by the methods described in Section 2.7.1.

TABLE 8.5

Analysed amino acid composition of the diets used in Experiment 6

(% dry matter basis)

Amino acids	DIET NO.									Requirement for carp[1]
	1	2	3	4	5	6	7	8	9	
Arginine	2.41	2.38	2.36	2.60	2.75	2.69	2.72	2.70	2.95	1.52
Histidine	0.84	0.85	0.84	0.86	0.84	0.84	0.85	0.84	0.83	0.56
Isoleucine	1.59	1.56	1.60	1.58	1.56	1.61	1.58	1.59	1.58	0.92
Leucine	2.85	2.88	2.82	2.80	2.84	2.80	2.78	2.78	2.80	1.64
Lycine	2.76	2.70	2.58	2.54	2.42	2.35	2.36	2.36	2.34	2.12
Methionine	1.09	1.08	1.02	0.96	0.95	0.85	0.86	0.84	0.84	0.64
Cystine	0.46	0.47	0.48	0.45	0.44	0.48	0.46	0.45	0.48	-
Phenylalanine	1.54	1.56	1.54	1.53	1.54	1.56	1.58	1.58	1.60	1.16
Tyrosine	1.10	1.12	1.10	1.04	1.02	0.99	0.98	0.96	0.98	-
Threonine	1.52	1.56	1.60	1.56	1.54	1.62	1.60	1.60	1.62	1.32
Valine	2.04	2.02	2.02	2.04	2.00	2.02	2.00	2.04	2.02	1.16
Alanine	2.31	2.33	2.35	2.40	2.36	2.16	2.14	2.12	2.10	-
Aspartic acid	3.80	3.83	3.79	3.88	3.84	3.72	3.76	3.70	3.78	-
Glutamic acid	5.11	5.10	5.12	5.42	5.46	5.84	5.80	5.82	5.94	-
Glycine	2.24	2.23	2.26	2.28	2.26	2.16	2.14	2.12	2.10	-
Proline	1.78	1.76	1.78	1.82	1.80	2.05	2.00	1.98	1.96	-
Serine	1.30	1.34	1.37	1.41	1.40	1.48	1.46	1.44	1.45	-

[1] Data for EAA requirement of carp from Ogino (1980)

TABLE 8.6

A summary of the methodology used to evaluate the oilseed meals as substitute for fish meal in the diet of carp

Fish	C. carpio, average initial weight 2.67±0.02g
Duration of Experiment	Eight weeks
Treatments	Substitution of fish meal by mustard oilcake, linseed and sesame meal in various combination in the diet of common carp
No. of Treatment	Nine
Replication	3/treatment
Water Temperature	27°C±1°C
Stocking Density	12/tank
Feeding Rate	3 times daily, 6% of the body weight
Flow Rate	1 litre/minute
Faeces Collection	Collected twice daily for two weeks, dried at 60°C in an oven and kept in air-tight container for subsequent chemical analysis
Carcass Sampling	Initial sample - 10 fish at the start of the experiment Final sample - 20 fish per treatment (5 fish from each replicate)
Physico-chemical characteristics monitored	Temperature, pH, dissolved oxygen, and total ammonia

respectively. Details of diet formulation and preparation were as described in Section 2.2.4 and 2.2.5 respectively. All the diets were analysed for proximate and amino acid composition and the results are presented in Table 8.4 and 8.5 respectively.

8.2.4. General Experimental Procedure

There were three replicates per treatment with 12 fish in each replicate of mean initial weight 2.67±0.02g. Acclimation and weighing procedures were as described earlier (Section 2.2.3). During acclimation fish were fed trout pellets (Ewos Baker's Omega No.3, 49% protein). Weighing of the fish during the experiment was carried out every seven days, after 12 hours of starvation. The experiment was conducted for a period of eight weeks. A summary of the methodology used in the present study is given in Table 8.6.

8.2.5. Feeding Rates

The fish were fed three times daily between 09.00 and 17.00 hours at four hourly intervals. Fish in each tank were fed 6% of their body weight per day. As moisture contents of all diets were similar and low (Table 6.4), no correction of feeding rate was made for moisture content. Details of food administration are given in Section 3.2.4. A record of the amount of food fed was kept for subsequent calculation of food conversion and protein utilization. The quantity of food per day was adjusted after each weekly weighing and fed for the subsequent week.

8.2.6. Faeces Collection

Fish were transferred to the faecal collection system (Section 2.1.2) at the beginning of the seventh week and faeces were collected twice daily, morning and evening, for two weeks. Details of the faeces collection procedure are given in Section 3.2.5.

8.2.7. Histological Techniques

Histological techniques were as described in Section 3.2.7. Twelve fish per treatment were sampled for histopathology to assess changes in gills, thyroid, liver, muscle, kidney and intestine.

8.2.8. Analysis of Experimental Data

Experimental results were analysed as described in Section 2.8.1. Statistical analyses were performed as described in Section 2.9.1.

8.3 RESULTS

8.3.1. Analysed Composition of the Diets

Proximate composition of the experimental diets is shown in Table 8.4. The moisture contents were very similar and varied between 5.00% and 5.87%. Protein levels were also similar and ranged from 39.95% to 41.17%. Lipid, crude fibre and ash content showed little variation. Anti-nutritional factors in the diets were calculated from their levels in the various plant proteins determined by the methods described in Section 2.7.1. Anti-nutritional factors showed marked variation between

diets according to the level of plant protein included in the respective diets.

8.3.2. Growth

Growth responses of carp fed the experimental diets are presented as initial and final mean weight, weight gain and specific growth rate (SGR) in Table 8.7 and graphically in Figure 8.1. It appears that the growth responses were significantly affected by the levels of plant protein inclusion. The fish meal based Control diet produced significantly ($p < 0.05$) the highest growth response throughout the experimental period, whilst Diet 9 (40% plant protein inclusion) resulted in the poorest growth (Figure 8.1).

Of the various combinations of plant proteins tested, Diet 2 containing 25% plant protein (equally contributed by mustard, linseed and sesame meal) resulted in significantly ($p < 0.05$) better growth performance than the other diets. At both 25% and 40% plant protein inclusion levels diets (5 and 9) containing higher levels of sesame meal produced significantly ($p < 0.05$) the poorest growth performances.

SGRs for different dietary groups ranged from 2.97 to 3.34. The SGR for the Control diet was significantly ($p < 0.05$) higher than for the rest of the diets. Diet 2 produced significantly ($p < 0.05$) the highest SGR among the plant protein diets. However, there were no significant ($p > 0.05$) differences between the SGRs of Diets 5, 6 and 8 and 5, 6 and 9.

TABLE 8.7

Growth and food utilization of common carp fed the experimental diets for eight weeks

	DIET NO.									± S.E.[2]
	1	2	3	4	5	6	7	8	9	
Initial weight (g)	2.68[a1]	2.69[a]	2.66[a]	2.66[a]	2.69[a]	2.70[a]	2.69[a]	2.66[a]	2.64[a]	0.02
Final weight (g)	17.40[a]	16.50[b]	15.94[c]	15.26[d]	14.35[fg]	14.68[ef]	14.97[de]	14.46[f]	13.96[g]	0.14
Weight gain (g)	14.72[a]	13.81[b]	13.28[c]	12.60[d]	12.66[fg]	11.98[ef]	12.28[de]	11.80[f]	11.32[g]	0.13
% weight gain(g)	549.2[a]	513.5[b]	499.2[c]	473.7[d]	433.5[f]	443.7[f]	457.0[e]	443.6[f]	428.8[g]	3.14
SGR (% day)	3.34[a]	3.24[b]	3.19[c]	3.12[d]	2.99[fg]	3.02[fg]	3.06[e]	3.02[f]	2.97[g]	0.01
SGR as % of Control	100	97.0	95.5	93.4	89.5	90.4	91.6	90.4	88.9	-
FCR	1.50[a]	1.54[ab]	1.57[bcd]	1.56[bc]	1.64[ef]	1.62[cde]	1.60[ef]	1.64[ef]	1.66[f]	0.02
PER	1.66[a]	1.57[b]	1.57[b]	1.56[b]	1.50[c]	1.54[bc]	1.56[b]	1.53[bc]	1.49[c]	0.02
ANPU %	29.80[a]	28.34[b]	28.04[b]	27.65[bc]	26.22[de]	26.77[cd]	27.90[b]	26.58[cde]	25.65[e]	0.34

[1] Figures in the same row having the same superscripts are not significantly ($p > 0.05$) different

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

8.3.3. Food Conversion Ratio (FCR)

Mean food conversion ratios (FCRs) for various diets ranged from 1.50 to 1.60 and are presented in Table 8.7. The Control diet and Diet 2 had significantly ($p < 0.05$) the lowest FCRs among the experimental diets. Diets 3 and 4 were the next most efficient with FCRs of 1.57 and 1.56 respectively. However, there were no significant ($p > 0.05$) differences between the FCRs of Diets 2, 3 and 4; 3, 4 and 6; 3 and 6; 5, 6, 7 and 8 and 5, 7, 8 and 9 respectively.

8.3.4. Protein Utilization

Protein utilization efficiency was measured in terms of protein efficiency ratio (PER) and apparent net protein utilization (ANPU %) and is presented in Table 8.7. The protein efficiency ratios followed the same trend as FCRs with the fish meal based Control diet producing significantly ($p < 0.05$) the highest PER. However, there were no significant ($p > 0.05$) differences between the PERs of Diets 2, 3, 4, 6, 7 and 8 and 5, 6, 8 and 9.

Mean apparent net protein utilization for various diets varied from 25.65 to 29.80 (Table 8.7). Again, the Control diet produced significantly ($p < 0.05$) the highest ANPU (29.80). However, there were no significant differences between the ANPUs of Diets 2, 3, 4 and 7; 4, 6 and 8 and 5, 8 and 9.

8.3.5. Nutrient Digestibility

Apparent digestibility of the nutrients in the experimental diets was determined as earlier (Section 2.8.1) and is presented in Table 8.8. Apparent dry matter digestibility values for different dietary treatments ranged from 46.66% to 67.78% with the Control having the highest value followed by Diet 2. Apparent protein digestibility for all the diets was fairly high ranging from 82.13% to 86.05%. Again, the Control diet had the highest apparent protein digestibility. Apparent lipid digestibility ranged from 90.89% to 93.40%.

The average apparent amino acid digestibility (AAAD) values for various diets ranged from 85.1% to 89.0% (Table 8.8). The average AAAD values in various diets were higher than the apparent protein digestibility values of the respective diets.

8.3.6. Carcass Composition

Proximate carcass composition of fish at the start and at the end of the experiment is presented in Table 8.9. Fish fed Diet 9 produced significantly ($p < 0.05$) the highest (79.01%) carcass moisture and lowest (4.31%) lipid content. Fish fed Diets 1 (Control) and 2 had significantly ($p < 0.05$) the lowest moisture and highest lipid and protein content. However, there were no significant ($p > 0.05$) differences between moisture contents of fish fed Diets 3 and 4; 5 and 7 and 6 and 8 respectively. There were no significant ($p > 0.05$) differences between carcass protein contents of fish fed Diets 1, 2 and 3 but these values were significantly higher than for the rest of the

TABLE 8.8

Apparent dry matter, protein, lipid and amino acid digestibilities (%) for the experimental diets*

	DIET NO.								
	1	2	3	4	5	6	7	8	9
Dry matter	67.8	63.0	61.4	61.9	57.6	54.6	57.9	56.3	46.7
Protein	86.1	85.8	85.6	85.7	83.4	82.8	84.1	84.7	82.1
Lipid	93.4	92.1	92.4	92.2	91.8	91.7	92.1	92.4	90.9
Arginine	90.9	90.1	90.4	90.2	89.4	88.5	88.8	88.5	87.7
Histidine	88.1	89.8	89.1	89.8	87.3	88.1	88.1	86.9	85.2
Isoleucine	89.0	86.6	87.1	87.7	85.8	86.2	85.1	84.6	82.1
Leucine	90.5	89.7	89.8	88.8	88.7	88.5	88.5	88.2	87.0
Lysine	90.7	90.1	89.9	88.7	85.6	85.3	88.1	86.3	84.0
Methionine	95.3	94.5	94.3	94.4	93.6	92.5	92.6	92.2	91.1
Cystine	90.2	89.2	90.1	88.9	86.3	86.7	89.0	87.3	86.7
Phenylalanine	85.6	86.8	85.9	86.1	86.2	86.0	85.4	84.4	84.3
Tyrosine	89.4	86.5	87.9	86.1	84.6	84.4	83.7	82.7	83.7
Threonine	87.1	86.8	85.5	86.1	85.6	86.8	84.2	84.1	84.2
Valine	89.4	88.6	87.9	88.2	85.8	85.2	85.7	86.5	84.9
Alanine	88.8	89.3	88.2	89.0	89.0	87.7	88.0	87.4	84.8
Aspartic acid	87.3	88.7	88.3	88.4	87.5	86.8	85.4	85.3	81.2
Glutamic acid	90.0	90.4	89.6	90.5	88.9	88.7	88.3	88.9	87.6
Glycine	85.2	85.9	85.6	85.6	84.8	85.1	86.7	86.1	84.6
Proline	88.4	87.7	87.1	87.9	85.6	85.2	83.1	84.5	84.2
Serine	86.6	87.3	85.6	87.6	85.4	85.9	83.8	82.2	82.7
Average AAAD	89.0	88.8	88.4	88.5	87.1	86.9	86.7	86.3	85.1

*No statistical analysis was possible as the determinations were done on pooled samples

TABLE 8.9

Proximate carcass composition (% fresh matter basis) of fish samples at the start and end of the experiment

	INITIAL (all fish)	FINAL Diet No. 1	2	3	4	5	6	7	8	9	± S.E.[2]
Moisture	80.96	77.40^{a1}	77.57^{a}	77.93^{b}	78.08^{b}	78.13^{c}	78.43^{d}	78.11^{c}	78.48^{d}	79.01^{e}	0.08
Crude protein	13.14	14.70^{a}	14.65^{ab}	14.42^{bc}	14.46^{abc}	14.43^{cd}	14.23^{cd}	14.29^{cd}	14.20^{cd}	14.13^{d}	0.08
Crude lipid	3.07	5.20^{a}	5.09^{ab}	4.99^{bc}	4.84^{cd}	4.86^{c}	4.76^{d}	4.84^{c}	4.69^{d}	4.31^{e}	0.06
Ash	2.49	2.52^{a}	2.55^{a}	2.55^{a}	2.52^{a}	2.51^{a}	2.51^{a}	2.52^{a}	2.52^{a}	2.49^{a}	0.02
TOTAL	99.62	99.82	99.86	99.89	99.90	99.93	99.93	99.78	99.89	99.94	-

[1] Figures in the same row having some superscripts are not significantly ($p > 0.05$) different

[2] Standard error of treatment mean calculated from the residual mean square in the analysis of variance

diets. The carcass lipid content in different dietary groups ranged from 4.31% to 5.20%. There were no significant ($p > 0.05$) differences between the carcass ash contents of fish fed various diets which ranged from 2.49% to 2.55%.

8.3.7. General Health and Histological Examination

At the end of the experiment no abnormalities or physical deformities were observed in fish examined from the various dietary treatment groups. Histopathological examination of the various organs such as gills, liver, kidney, muscle, thyroid and intestine of fish from various dietary treatments revealed no significant changes in these tissues.

8.4 DISCUSSION

The results of the present investigation showed that substitution of fish meal by various plant protein sources in different combinations resulted in improved growth rate, food conversion and protein utilization compared to that of single plant proteins used at the same level.

The results obtained here are in good agreement with those of Hasan (1986) who reported better growth performance of carp fry fed diets containing linseed and groundnut meal; mustard linseed and sesame meal; and linseed, groundnut and sesame meal respectively, compared with the diets containing mustard, linseed, groundnut or sesame meals alone. This author also noted that a combination of 21% linseed, 14% groundnut

and 32% fish meal in a diet produced even better growth performance than that with fish meal based Control diet.

As already discussed in the Introduction (Section 8.1) several authors have reported comparatively better growth of fish fed diets containing combinations of plant protein sources (Olkunle, 1982; Richards, 1983). These authors found better growth performance of O. mossambicus when fed diets containing groundnut and sunflower meal; and sunflower and sesame meal respectively, as partial substitute for fish meal protein compared with diets containing groundnut, sesame or sunflower meal alone.

The effectiveness of using various combinations of ingredients in fish feed has also been reported by Tacon et al. (1984) who successfully reduced the fish meal level from 50% to 10% by soybean, meat and bone meal, brewers yeast, puffed maize and blood meal in the diet of tilapia (O. niloticus) without reducing the growth performance.

Of the various combinations tested, Diet 2 (in which 25% fish meal protein was replaced by equal amounts of mustard, linseed and sesame protein) produced significantly better weight gain among the plant protein containing diets. The weight gain was about 93.8% of that for the fish meal based Control diet. This growth performance was also better than that of 73% obtained in Experiment 1 (Section 3.3.5) using 25% mustard oilcake protein alone.

This better growth performance of fish fed Diet 2 could be attributed to the lower levels of anti-nutritional factors in Diet 2 compared to

Experiment 1. For example, Diet 2, in this study contained 0.034% allyl isothiocyanate and 0.16% tannins (Table 8.4) compared to that of 0.16% allyl isothiocyanate and 0.57% tannins in Diet 2 (25% mustard protein) of Experiment 1 (Table 3.5). The allyl isothiocyanate level of 0.034% in Diet 2, in this study, is within the tolerance limit of carp as has been observed in Experiment 5.2 (Table 7.5).

However, the phytic acid level (0.46%) in Diet 2 might be responsible for the slightly lower growth performances of fish compared to that in the fish meal based Control diet. Spinelli et al. (1983) reported that rainbow trout fed diets containing as low as 0.5% phytic acid had 10% reduced growth and feed conversion.

The growth performances of fish fed diets (6, 7, 8 and 9) in which 40% fish meal protein was substituted by various combinations of mustard, linseed and sesame meals also produced better growth performances than those obtained in Experiment 1 (Table 3.9) at 25% fish meal protein substitution levels using single plant protein sources.

None of the diets (6, 7, 8 and 9) at the 40% substitution level in this study were deficient in any EAA (Table 8.5). In contrast, diets, even at 25% plant protein substitution level, in Experiment 1 using linseed and sesame meal as single plant protein sources were deficient in some of the EAAs (Table 3.6). This clearly supports the views of Jackson et al. (1982) who advocated the use of different plant protein sources in combination as a means of compensating EAA deficiency in tilapia diets.

The levels of individual anti-nutritional factors in Diets 6, 7, 8 and 9 (Table 8.4) at 40% fish meal substitution level in this study were much lower than in the diets at the 25% substitution level in Experiment 1 (Table 3.5). Although the various combinations of plant proteins kept the individual anti-nutritional factors in diets comparatively low, the phytic acid and allyl isothiocyanate in Diets 6, 7, 8 and 9 (Table 8.4) were still high enough to cause growth depression (Higgs et al., 1982; Spinelli et al., 1983; Hilton and Slinger, 1986). Thus, the higher levels of phytic acid and allyl isothiocyanate in Diets 6, 7, 8 and 9 might be responsible for the lower growth performances of fish compared to the Control.

The FCRs obtained in this study (Table 8.7) were lower than those found in Experiment 1 (Table 3.9) and by Hasan (1986) in carp using similar oilseed meals. However, PERs revealed a pattern very like that of FCR (Table 8.7).

Data on apparent protein digestibility in all diets indicated good utilization of all protein mixtures and the values were similar to those reported for fish meal and soybean in tilapia (Shiau et al., 1987); fish meal and groundnut; fish meal, mustard, linseed and sesame meal in carp (Hasan, 1986) and fish meal and lupin meal in rainbow trout (De La Higuera et al., 1988).

The average apparent amino acid digestibility (AAAD) values found here were higher than the apparent protein digestibility (APD) values of the respective diets (Table 8.8). Similarly Vens-Cappell (1984) and Wilson

et al. (1981) also found higher average AAAD values than APD values in rainbow trout and channel catfish respectively. However, the AAAD values found in diets with various combinations of plant proteins in this study were slightly higher than obtained in Experiment 3 (Table 5.9) using mustard, linseed and sesame meal as single sources of protein.

The carcass composition was little affected by dietary treatments. However, diets with higher levels of plant proteins produced lower lipid and higher moisture contents probably as a consequence of the anti-nutritional factors in the oilseed meals.

The results of the present study demonstrated that the use of different plant protein sources in various combinations is more effective than that of a single source in the substitution of fish meal in carp diets. Use of different plant protein sources in various combinations can prevent a high inclusion level of any single anti-nutritional factor in the diet and can also be a means of compensating for essential amino acid deficiency in any single protein source.

CHAPTER 9 : GENERAL DISCUSSION AND CONCLUSION

9.1 GENERAL DISCUSSION

The aim of the research project was to investigate the nutritional suitability of some Bangladeshi oilseed by-products such as mustard oilcake, linseed and sesame meal as protein sources to substitute fish meal in the diet of common carp. Investigations included:

- quantification of the major anti-nutritional factors present in these oilseed meals;
- determination of nutrient digestibility coefficients for these oilseed meals;
- study of the possible toxic effects of these anti-nutritional factors on fish growth and health;
- detoxification of these oilseed meals using various processing treatments;
- use of synthetic anti-nutritional factors in purified diets to determine their effects on growth performances as compared with diets containing the same quantity of anti-nutritional factors from the ingredients under test.

Mustard oilcake, linseed and sesame meals are the main oil crops in Bangladesh (Table 1.6). These oilcakes and meals are traditional, and highly valued, feeds for farm animals. The average protein content in these oilseed meals varies between 35% to 45%. Both linseed and sesame meals are relatively high in the essential amino acid methionine but low in lysine.

Because of limited supply, and high price, the use of fish meal as the sole or major source of protein in formulated diets for carp fry and fingerlings in most developing countries, including Bangladesh, is not feasible.

In Bangladesh the cost of protein (Tk/kg of protein*) using fish meal as the source is Tk. 22.81/kg, whereas it is Tk. 5.70, 8.70 and 12.45 per kg from sesame, linseed and mustard oilcakes respectively (Hasan, 1986). Therefore the use of alternative protein sources as partial substitutes for fish meal in the formulation of diets for carp fry and fingerlings is worthy of particular or special attention.

Of the plant protein sources tested as partial substitutes for fish meal (Chapter 3, Experiment 1), at various protein substitution levels (e.g. 25%, 50% and 75% of total protein), linseed meal was the best followed by mustard oilcake at 25% inclusion.

The practice of carp polyculture in earthen ponds has gained prominence in India and Bangladesh in recent years. In order to support higher stocking densities, supplementary feeds are given. These feeds consist of mixtures of rice bran and mustard oilcake (1:1 ratio) given at a rate of 1% to 4% body weight of fish (ADCP, 1983). Sometimes dry oilcake is soaked in water and usually sprayed or sprinkled over the pond water surface.

Although the use of mustard oilcake in combination with rice bran has

---

*45 Bangladeshi Taka = 1 UK pound Sterling

been reported to increase fish production significantly (Jhingran, 1977), its precise value as a fish feed has never been established. It is not unlikely that a considerable proportion of these ingredients act as pond fertilizers rather than being used directly by the fish. The results of the present investigation indicate that mustard oilcake is unsuitable as a dietary protein source at a level of 25% of the total protein, or more, without sacrificing fish growth.

All the diets containing plant proteins, except Diet 2 (25% mustard protein) in Experiment 1, were deficient in one or more essential amino acid (EAA). Although the growth depression of fish in Experiment 1 was believed to be principally due to the higher levels of anti-nutritional factors (see Table 3.5), Experiment 2 was conducted to determine whether supplementation of dietary crystalline EAA improved their nutritive value to carp.

Reports on the utilization of supplemental crystalline amino acids are contradictory among fish species (Aoe et al., 1970; Andrews and Page, 1974; Murai et al., 1981, 1982a; Robinson et al., 1982). However, the results of Experiment 2 demonstrated that carp can utilize crystalline EAA. Furthermore, the growth improvement was more pronounced in fish fed diets supplemented with all the deficient EAA than in fish fed diets supplemented with the first limiting EAA. However, although supplementation of EAA improved the growth performance, the growth was not equivalent to that of the fish meal based Control diet. This may be attributed to the presence of anti-nutritional factors in the plant protein sources.

Since the nutritive value of protein is influenced by the physiological availability of its constituent amino acids, levels of amino acids determined chemically may not adequately reflect biological availability (Austic, 1983). As there is no published information on the amino acid availability of feed ingredients for carp, Experiment 3 (Chapter 5) was conducted to study the protein, energy and amino acid digestibilities of fish meal, mustard oilcake, linseed and sesame meal.

An indirect method successfully applied to terrestrial animals has been used for this digestibility study. The method is based on the assumption that the amount of chromic oxide in feed and faeces remain constant over the experimental period. Its advantage over direct methods is elimination of the need for quantitative collection of faeces.

Faeces collected after being voided into water may suffer considerable leaching leading to overestimation of digestibility (Windell et al., 1978a; Choubert et al., 1979). In the present study, considering the small size of the experimental fish, a specially designed tank system was used for faeces collection.

The results of Experiment 3 (Chapter 5) showed that apparent protein digestibility (APD) values of mustard oilcake, linseed and sesame meal are fairly high and are generally in agreement with the reported APD values of various oilseed meals for fish (see Table 5.11). Except for sesame meal, the apparent amino acid digestibility (AAAD) values of linseed, mustard oilcake and fish meal were similar to their APD values

suggesting that the protein digestibility values are indicative of amino acid digestibility.

As discussed in Chapter 6, oilseed meals contain glucosides which, upon hydrolysis by endogenous enzymes, release substances that may be toxic (Liener, 1977). Suitable processing techniques, such as aqueous treatment, heat treatment or autoclaving, have been reported to increase the feeding value of mustard oilcake by destroying or reducing the enzymes thereby preventing enzymatic breakdown of glucosinolates to isothiocyanates (Tookey et al., 1980). Capper et al. (1982) observed that fingerling common carp fed a diet containing 20% roasted mustard meal from Nepal did not suffer depressed growth or decreased food conversion.

Experiment 4.1 (Chapter 6) was conducted to evaluate detoxification of mustard oilcake by a variety of processing techniques such as heat, aqueous and enzyme treatments to reduce or destroy the anti-nutritional factors (e.g. allyl isothiocyanate and tannins) and to evaluate the nutritive value of detoxified mustard oilcake. Both aqueous and enzyme treatments were effective in reducing toxic factors in mustard oilcake (Table 6.3). In Bangladesh, the expeller method is the most commonly used for oil extraction while commercial heat treatment or enzyme treatment of expeller cake can be expensive or difficult to operate under field conditions. However, aqueous treatment of mustard oilcake may be cheap and feasible under field conditions.

In Experiment 4.2 (Chapter 6) detoxification of linseed and sesame meals

was accomplished by water extraction and heat treatment. Phytic acid is the main anti-nutritional factor in both linseed and sesame meal. In addition linseed also contains cyanogenic glucosides which release HCN upon hydrolysis by an enzyme. The results of the study indicated that heat treatment was more effective in reducing the phytic acid while aqueous treatment was effective in reducing HCN in linseed meal.

Phytic acid reduces the nutritive value of a product either by forming complexes with protein or by reacting with calcium, magnesium, copper, iron or zinc in the food and thereby inhibiting the absorption of these minerals (Hartman, 1979). Heat treatment or autoclaving reduces the ability of phytic acid to bind metal ions or other compounds thereby improving the nutritive value of food.

Although the adverse effects of phytic acid in the presence of high dietary calcium on growth and mineral bioavailability in rats, poultry and swine are conclusive, reports so far on fish are controversial (Spinelli et al., 1983; Richardson et al., 1985).

Experiment 5.1 (Chapter 7) was conducted to study the effects of phytic acid in purified diets containing different levels of calcium and magnesium on growth and mineral bioavailability in carp. The results of the investigation confirmed the findings of Richardson et al. (1985) that dietary phytic acid depressed growth and mineral bioavailability in chinook salmon.

Increasing calcium and magnesium levels in the presence of phytic acid

significantly reduced growth and mineral bioavailability as reflected by these elements in plasma, liver, kidney and carcass. The pathological effects of phytic acid were characterized by hypertrophy and marked vacuolization of the cytoplasm of the epithelial cells in the intestine of fish fed Diet 9 containing the highest level of phytic acid and calcium and magnesium.

The growth depressing and toxic effects of the glucosinolates of rapeseed and canola meal in rainbow trout and chinook salmon have been reported by various authors (Yurkowski et al., 1978; Higgs et al., 1982; Hardy and Sullivan, 1983; Hilton and Slinger, 1986). However, the toxic effects of the glucosinolates of mustard oilcake on fish have not yet been reported. Experiment 5 (Chapter 7) was conducted to study the toxic effects of glucosinolate (allyl isothiocyanate) on growth and histopathology of carp.

The results of the study indicated that the tolerance limit to dietary glucosinolate (allyl isothiocyanate) in carp is less than 0.4 mg/g of diet. However, this level is slightly higher than that of 0.3mg total glucosinolate/g diet for chinook salmon (Higgs et al., 1982) and 0.23-0.34mg total glucosinolate/g diet for trout (Hardy and Sullivan, 1983). The variation in the tolerance level of glucosinolates between carp, trout and salmon may be due to species specific responses (Dabrowski et al., 1982) as well as the types of glucsinolate used by various authors. For example, the major glucosinolate in mustard oilcake is allyl isthiocyanate whereas it is butenyl isothiocyanate in rapeseed meal.

The toxic effects of glucisonlate were also reflected in fish fed diets containing higher levels of mustard oilcake or synthetic allyl isothiocyanate. The most significant abnormal changes were in the thyroid tissues, both in the pharyngeal and kidney tissues.

In terms of dietary protein utilization, carp fed diets containing untreated plant protein (Chapters 3, 4, 6 and 8) achieved PER and ANPU values which were below those recorded for fish fed the fish meal based Control diet. In general, apparent protein digestibility decreased with the increase of level of plant protein in the diet. The reduced growth performance and food utilization in these diets was attributed to the anti-nutritional factors in plant proteins. The incorporation of various protein sources has often resulted in a hitherto unexplained reduction in carcass lipid and increase in moisture content.

In view of the depressed growth performance of fish fed diets containing single plant protein sources (Chapters 3 and 6), an attempt was made in Experiment 6 to use various combinations of plant protein sources to substitute fish meal. The results of the study showed that the use of plant proteins in various combinations is more effective than that of single sources in the substitution of fish meal in carp diets. Furthermore, the use of different protein sources in various combinations can prevent a high inclusion of any single anti-nutritional factor and act as a means of compensating for EAA deficiency in a single protein source.

The growth performance of fish fed Diet 2, containing a combination of

plant protein sources, in Experiment 6 (Chapter 8) was comparable to that obtained in fish fed diets containing detoxified oilseed meals (Experiment 4.1 and 4.2). Therefore the use of various combinations of plant proteins can be useful where commercial processing or treatment of oilseed meals are expensive or difficult.

9.2 SUGGESTIONS FOR FUTURE WORK

The results of the present investigation demonstrated that mustard oilcake, linseed and sesame meal cannot be used as partial substitute for fish meal in carp diets at levels of 25% dietary protein or more without sacrificing growth and food utilization.

Although the toxic effects of glucosinolate in mustard oilcake on carp has been tested in this study, there is no information on the toxicity of tannins to fish. As already mentioned in Section 1.4.4, tannins are reported to have adverse effects on growth and protein utilization in poultry (Change and Fuller, 1964; Vohra et al., 1966; Marquardt et al., 1977). Therefore further studies should be undertaken on the toxic effects of tannins (in mustard oilcake) to carp.

Information on the possible effects of linamarin (in linseed) on fish are lacking. Long term studies should be undertaken on the possible effects of linamarin on the growth and health of carp. Further investigation should include studies on the effects of the above mentioned plant protein sources on the digestive enzyme (e.g. Trypsin, Chymotrypsin, Pepsin, Carboxypeptidase and Amylase) activity of carp.

It is possible that the young carp used in this investigation are more sensitive to the anti-nutritional factors than larger fish would be. Therefore, long term studies should be conducted using fish over a larger size range than those used in the present study.

9.3 CONCLUSIONS

1. The protein contents of mustard oilcake, linseed and sesame meal used in this investigation were fairly high and varied from 32.74% to 36.97%; 32.96% to 33.74% and 36.69% to 43.09% respectively.

2. The anti-nutritional factors in these oilseed meals varied as follows:

(i)	Glucosinolate (allyl isothiocyanate)	0.36-0.54%
(ii)	Phytic acid	
	Linseed meal	2.45-2.64%
	Sesame meal	2.38-2.86%
(iii)	Tannins	1.64-1.87%
(iv)	Hydrocyanic acid (HCN)	0.032-0.043%

3. The results of evaluation of these plant proteins as partial substitutes for fish meal in carp diets, using them as single sources, demonstrated that they cannot be used at a level of 25% or more of the dietary protein without sacrificing growth and feed efficiency.

4. An investigation on the effects of EAA supplementation in plant protein diets demonstrated that carp can utilize crystalline amino acids and supplementation of EAA improved the nutritive value of diets containing plant proteins.

5. The growth performance was more pronounced in fish fed diets supplemented with all deficient EAA than in fish fed diets supplemented with the first limiting EAA alone.

6. Results from an experiment to study the nutrient digestibility suggest a reasonable agreement between protein digestibility and average amino acid digestibility. In general, the protein digestibility values were indicative of amino acid digestibility.

7. However, because of variation within individual amino acid digestibilities in a feed ingredient, the use of specific amino acid digestibility value would allow more accurate and economic feed formulation.

8. From the nutrient digestibility viewpoint it appears that carp may be able to utilize mustard, linseed and sesame meals efficiently as dietary protein sources.

9. The results of the detoxification of mustard oilcakes by various processing treatments indicated that both aqueous and enzyme treatments were effective in reducing the anti-nutritional factors. For example, aqueous and enzyme treatment reduced about 49% and 57% of

allyl isothiocyanate and 30% and 32% of tannins in mustard oilcakes respectively.

10. In the case of linseed and sesame meals heat treatment was found to be more effective in destroying anti-nutritional factors. Heat treatment reduced by about 72% and 74% of the phytic acid levels in linseed and sesame meal respectively.

11. Use of detoxified mustard oilcake, linseed and sesame meals in diets improved the growth performance and food utilization in carp compared to the untreated meals.

12. Investigation on the effects of dietary phytic acid demonstrated that phytic acid as low as 0.5% depressed growth, food utilization and mineral bioavailability (especially calcium and zinc) in carp. Phytic acid in the presence of increasing dietary calcium and magnesium levels significantly influenced the growth and mineral (especially calcium, magnesium, zinc, iron and copper) bioavailability.

13. This reduced growth and mineral bioavailability is believed to be due to the formation of insoluble protein-phytate complex and/or phytate mineral complex.

14. Experiments on the effect of dietary glucosinolate (allyl isothiocyanate) on growth and food utilization demonstrated that carp can tolerate 0.4mg glucosinolate/g diet without inhibiting the growth performance or adverse effects on fish health.

15. Fish fed diets containing higher levels of mustard oilcake or allyl isothiocyanate showed abnormal changes in thyroid tissues. Abnormalities in thyroid tissues indicate an inhibition of thyroid hormone production. These adverse effects have been attributed to the isothiocyanate formed from glucosinolate.

16. The results of an evaluation of oilseed meals demonstrated that the use of various plant proteins in combination is more effective than that of a single source in the substitution of fish meal in carp diets. Use of different plant protein sources in various combinations can prevent a high inclusion of single anti-nutritional factors in the diet.

REFERENCES

REFERENCES

ABEL, H., BECKER, K., MESKE, C. and FRIEDRICH, W., 1984. Possibilities of using heat treated full-fat soybean in carp feeding. Aquaculture, 42: 97-108.

AGREN, G. and EKLUND, A., 1972. Rapeseed protein fractions. III. The nutritive value of a detoxified protein concentrate prepared from rapeseed (Brassica napus L) by hydraulic processing. J. Sci. Food Agric., 23: 1457-1462.

ALI, M. L., 1985. Inland Fisheries Resources. Present Status and Future Potential in Bangladesh. Paper presented in the National Seminar, 85, on Fisheries Development in Bangladesh. Dhaka, 15-19 January.

ALMQUIST, H. J. and GRAU, C. R., 1944. Mutual supplementary effect of the proteins of soybean and sesame meals. Poult. Sci., 23: 341-343.

ANDERSON, R. J., 1914. A contribution to the chemistry of phytin. J. Biol. Chem., 17: 171-174.

ANDREWS, J. W. and STICKNEY, R. R., 1972. Interactions of feeding rates and environmental temperature on growth, food conversion and body composition of channel catfish. Trans. Am. Fish. Soc., 101: 94-99.

ANDREWS, J. W. and PAGE, J. W., 1974. Growth factor in the fish meal component of catfish diets. J. Nutr., 104: 1091-1096.

AOE, H., MASUDA, I., ABE, I., SAITO, T., TOYODA, T. and KITAMURA, S., 1970. Nutrition of protein in young carp - I. Nutritive value of free amino acids. Bull. Jap. Soc. Sci. Fish., 36: 407-413.

AOE, H., IKEDA, K. and SAITO, T., 1974. Nutrition of protein in young carp - II. Nutritive value of protein hydrolyzates. Bull. Jap. Soc. Sci. Fish., 40: 375-379.

APPELQVIST, L. A. and JOSEFFSON, E., 1967. Method for quantitative determination of isothiocyanates and oxazolidinethiones in digest of seed meal of rape and turnip rape. J. Sci. Food Agric., 18: 510-519.

APPLER, H. N., 1984. Green algae as protein source for Oreochromis niloticus and Tilapia zillii. PhD Thesis, Stirling University, 243pp.

APPLER, H. N., 1985. Evaluation of Hydrodictyon reticulatum as protein source in feeds for Oreochromis (Tilapia) niloticus and Tilapia zillii. J. Fish. Biol., 27: 327-334.

APPLER, H. N. and JAUNCEY, K., 1983. The utilization of a filamentous green algae (Cladophora glomerata) as protein source in pelleted feeds for Sarotherodon (Tilapia) niloticus fingerlings. Aquaculture, 30: 21-30.

AQUATIC FARMS, 1986. Bangladesh Second Aquaculture Development Project Preparation Report. Submitted to the Peoples Republic of Bangladesh and the Asian Development Bank, February 1986.

AQUACULTURE DEVELOPMENT AND COORDINATION PROGRAMME, 1983. Fish feeds and feeding in developing countries. ADCP/REP/83/18. UNDP, FAO, Rome. 97pp.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 1980. Official methods of analysis of the Association of Official Analytical Chemists. (ed. W. Horwitz), 13th edition. Washington DC, 1018pp.

ATACK, T. H., JAUNCEY, K. and MATTY, A. J., 1979. The utilization of some single cell proteins by fingerling mirror carp (Cyprinus carpio). Aquaculture, 18: 337-348.

ATACK, T. H. and MATTY, A. J., 1978. The evaluation of some single cell proteins in the diet of rainbow trout. II. The determination of net protein utilization, biological value and true digestibility. Symp. Fin-fish Nutr. and Feed Technol., Hamburg, FRC, 20th June 1978. EIFAC/78/Symp. E/59, FAO, Rome,

ATKINSON, J. L., HILTON, J. W. and SLINGER, S. J., 1984. Evaluation of acid insoluble ash as an indicator of feed digestibility in rainbow trout (Salmo gairdneri). Can. J. Fish. Aquat. Sci., 41: 1384-1386.

AUSTIC, R. E., 1983. The availability of amino acids as an attribute of feeds. In: Feed Information and Animal Production (G. E. Robards and R. G. Packham, eds): 175-189. Commonwealth Agricultural Bureau.

AUSTRENG, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture, 13: 265-172.

BALLESTER, D., RODRIGO, R., NAKOUZI, J., CHICHESTER, C. O., YANEZ, E., and MONCKEBERG, F., 1970. Rapeseed meal - III. A simple method for detoxification. J. Sci. Food Agric., 21: 143-144.

BALLESTER, D., RODRIGUEZ, B., RAJAS, M., REID, O. B. A., YANEZ, E. and MONCKEBERG, F., 1973. Rapeseed meal - IV. Continuous water extraction and short term feeding studies in rats with the detoxified product. J. Sci. Food Agric., 24: 127-138.

BANGLADESH BUREAU OF STATISTICS, 1984. Statistical year book of Bangladesh, 1983-1984. Bangladesh Bureau of Statistics, Dhaka, 138pp.

BANGLADESH FISHERY RESOURCES SURVEY SYSTEM, 1984. Assessment of Water Area and Fish Production in Bangladesh (MS). Bangladesh Fishery Resources Survey System (FAO Project BGD/79/015), 3pp.

BARNES, R. K. and MANN, K. H., 1980. Fundamentals of Aquatic Ecosystem. Blackwell Scientific Publications, Oxford, London, 229pp.

BELL, J. M. and BELZILE, R. J., 1965. Goitrogenic properties. In: Rapeseed meal for livestock and poultry - review. (G. P. Bowland, D. R. Clardinin and L. R. Wetter, eds): 33-46. Canada Department of Agriculture.

BELL, J. M., DOWNEY, R. K. and WETTER, L. R., 1963. Oil and meal from Canadian rapeseed. Canada Department of Agriculture Publication 1183.

BELL, J. M., SHIRES, A., BLAKE, J. A., CAMPBELL, S. and MCGREGOR, D.I., 1981. Nutritional evaluation of ammoniated mustard meal for use in swine feeds. Can. J. Anim. Sci., 64: 1023-1033.

BELZILE, R., BELL, J. M. and WETTER, L. R., 1963. Growth depressing factors in rapeseed oil meal. V. The effects of myrosinase activity on the toxicity of the meal. Can. J. Anim. Sci., 43: 169-173.

BELZILE, R. J. and BELL, J. M., 1966. Growth depressing factors in rapeseed oil meal. VII. Effects of myrosinase activity on toxicity following treatment with buffered solutions. Can. J. Anim. Sci., 46: 165-169.

BELZILE, R. J., POLQUIN, L. S. and JONES, J. D., 1974. Nutritive value of rapeseed flour for mink. Effect on live performance, nutrient utilization, thyroid function and pelt quality. Can. J. Anim. Sci., 54: 639-644.

BERGSTROM, E., 1979. Experiments on the use of single cell proteins in Atlantic salmon diets. In: Finfish Nutrition and Fish Feed Technology. (J. E. Halver and K. Tiews, eds), Vol. II: 105-116. H. Heenemann GmbH, Berlin.

BILLIE, N., EGGUM, B. O., JACOBSEN, I., OLSEN, O. and SORENSEN, H., 1983. Antinutritional and toxic effects in rats of individual glucosinolates (±myrosinase) added to a standard diet - I. Effect on protein utilization and organ weights. Z. Tierphysiol., Tierernahrg, u. Futtermittelkde, 49: 195-200.

BOWEN, S. H., 1981. Digestion and assimilation of periphytic detrital aggregate by Tilapia mossambica. Trans. Am. Fish. Soc., 110: 239-245.

BROWN, M. E., 1957. Experimental studies on growth. In: The Physiology of Fishes. (M. E. Brown, ed), Vol.I: 361-400. Academic Press, New York.

BROWN, P. B., STRANGE, R. J. and ROBBINS, K. R., 1985. Protein digestibility coefficient for yearling channel catfish fed high protein feedstuffs. Prog. Fish Cult., 47: 94-97.

BRYANT, P. L., 1980. The Husbandry and Nutrition of Carp Larvae and Fry. PhD Thesis, University of Aston in Birmingham, 211pp.

BUDDINGTON, R. K., 1979. Digestion of an aquatic macrophyte Tilapia zillii (Gervais). J. Fish. Biol., 15: 449-455.

BUDDINGTON, R. K., 1980. Hydrolysis-resistant organic matter as a reference for measurement of fish digestive efficiency. Trans. Am. Fish. Soc., 109: 653-656.

BYRD, C. N. and MATRONE, G., 1965. Investigation of chemical basis of zinc-calcium-phylate interaction in biological systems. Proc. Soc. Expt. Biol. Med., 119: 347-349.

CAPPER, B. S., WOOD, J. F. and JACKSON, A. J., 1982. The feeding value for carp of two types of mustard seed cake from Nepal. Aquaculture, 29: 373-377.

CHAKRABARTY, R. D., SEN, P. R., CHATTERJEE, D. K. and KOWTAL, G. V., 1973. Observations on the relative usefulness of different feed for carp spawn and fry. J. Inland Fish. Soc. India, 5: 182-188.

CHANG, R., SCHWIMMER, S. and BURR, H. K., 1977. Phylate: Removal from whole dry beans by enzymatic hydrolysis and diffusion. J. Food Sci., 42: 1098-1101.

CHANG, S. I. and FULLER, H. I., 1964. Effect of tannin content of grain sorghums on their feeding value for growing chicks. Poult. Sci., 43: 30-36.

CHAUDHURI, H., CHAKRABORTY, R. D., SEN, P. R., RAO, N. G. S. and JANA,S., 1975. A new high in fish production in India with record yield by composite fish culture in fresh water ponds. Aquaculture, 6: 343-355.

CHERYAN, M., 1980. Phytic acid interactions in food systems. CRC crit. Rev. Food Sci. Nutr., 13: 297-335.

CHIU, Y. N., CAMACHO, A. S. and SASTRILLO, M. A. S., 1986. Effect of amino acid supplementation and vitamin level on the growth and survival of milkfish (Chanos chanos) fry: 543-546. In: J. L. Maclean, L. B. Dizon, and L. V. Hosillos (eds). The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

CHO, C. Y. and SLINGER, S. J., 1979. Apparent digestibility measurements in feedstuffs for rainbow trout. In: Finfish Nutrition and Fish Feed Technology. (J. E. Halver and K. Tiews, exs), Vol.II: 239-247. H. Heenemann GmbH, Berlin.

CHO, C. Y., SLINGER, S. J. and BAYLEY, H. S., 1982. Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. Comp. Biochem. Physiol., 73.B: 25-41.

CHOUBERT, G., De La NOUE, J. and LUQUET, P., 1979. Continuous qualitative automatic collector for fish faeces. Prog. Fish Cult., 41: 64-67.

CHOUBERT, G., De La NOUE, J. and LUQUET, P., 1982. Digestibility in Fish: Improved device for the automatic collection of faeces. Aquaculture, 29: 185-189.

CHOWDHARY, S. K., SRIVASTAVA, J. B. and IQBAL, S. E., 1978. Observations on the relative usefulness of different feeds for common carp (Cyrpinus carpio var. communis Linn). Matsya, 4: 20-24.

CHUA, T. E., 1986a. Aquaculture production in Asia: a reassessment. Naga: ICLARM Q9(2): 13-15.

CHUA, T. E., 1986b. An overview of the fisheries and aquaculture industries in Asia: 1-8. In: J. L. Maclean, L. B. Dizon and L. V. Hosillos (eds) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

CLANDININ, D. R. and BAYLY, L., 1960. Effect of feeding rapeseed oil meal on the structure of thyroid gland of chickens. Poult. Sci., 39: 1239-1240.

CLANDININ, D. R., BAYLY, L. and CABALLERO, A., 1966. Effect of (±)-5-vinyl-2-oxazolidinethione, a goitrogen of rapeseed meal, on the rate of growth and thyroid function of chicks. Poult. Sci., 45: 833-838.

CLANDININ, D. R. and HEARD, J., 1968. Tannins in prepress-solvent processed rapeseed meal. Poult. Sci., 47: 688-671.

COURSEY, D. G., 1973. Cassava as food: toxicity and technology: 27-36. In: Chronic cassava toxicity: proceedings of an inter-disciplinary workshop, London, England, 29-30 January 1973. Int. Develop. Res. Centre Monogr. IDRC-010e.

DABROWSKA, H. and WOJNO, T., 1977. Studies on the utilization by rainbow trout (Salmo gairdneri) of fed mixtures containing soybean meal and an addition of amino acids. Aquaculture, 10: 297-310.

DABROWSKI, K. and KOZAK, B., 1979. The use of fish meal and soybean meal as a protein source in the diet of grass carp. Aquaculture, 18: 107-114.

DABROWSKI, K. and KOZLOWSKA, H., 1981. Rapeseed meal in the diet of common carp reared in heated waters. I. Growth of fish and utilization of diet. In: K. Tiews (ed), Proc. World Symp. on Aquaculture in Heated Effluents and Recirculation Systems, Vol.II: 263-274.

DABROWSKI, K., EVANS, R., CZAROCKI, J. and KOZLOWSKA, H., 1982. Rapeseed meal in the diet of common carp reared in heated waters. IV. Iodide (^{125}I) accumulation and thyroid histology. Z. Tierphysiol. Tierernahrg. u. Futtermittelkde, 48: 1-9.

DAVIES, N. T. and NIGHTINGALE, R., 1975. The effects of phytate on intestinal absorption and secretion of zinc and whole-body retention of zinc, copper, iron and manganese in rats. Br. J. Nutr., 34: 243-258.

DEAN, W. F. and SCOTT, H. M., 1965. The development of an amino acid reference diet for the early growth of chicks. Poult. Sci., 44: 803-808.

DE LA HIGUERA, M., GARCIA-GALLEGO, M., CARDENETE, G., SUAREZ, M. D. and MOYANO, F. J., 1988. Evaluation of lupin seed meal as an alternative protein source in feeding of rainbow trout (Salmo gairdneri). Aquaculture, 71: 37-50.

DE LA NOUE, J. and CHOUBERT, G., 1986. Digestibility in rainbow trout: Comparison of the direct and indirect methods of measurement. Prog. Fish Cult., 48: 190-195.

DE SILVA, S. S. and PERERA, M. K., 1983. Digestibility of an aquatic macrophyte by the cichlid Etroplus suratensis (Bloch) with observations on the relative merits of these indigenous components as markers and daily changes in protein digestibility. J. Fish Biol., 23: 675-684.

DICKSON, M. W., 1981. Assessment of six different feeds for Sarotherodon sp in Kenya. MSc Thesis, University of Stirling. 70pp.

DORSA, W. J., ROBINNETTE, H. R., ROBINSON, E. H. and POE, W. E., 1982. Effects of dietary cottonseed meal and gossypol on growth of young channel catfish. Trans. Am. Fish. Soc., 111: 90-93.

DROULISCOS, N. J. and BOWLAND, J. P., 1969. Biological evaluation of rapeseed meal, soybean meal and casein fed to the weanling and the mature rats. Br. J. Nutr., 23: 113-123.

DUNCAN, D. B., 1955. Multiple Range and Multiple F Tests. Biometrics, 11: 1-42.

EAPEN, K. E., TAPE, N. W. and SIMS, R. P. A., 1968. New process for the production of better-quality rapeseed oil and meal. I. Effect of heat treatments on enzyme destruction and color of rapeseed oil. J. Am. Oil Chem. Soc., 45: 194-19.

ERDMAN, J. W., Jr., 1979. Oilseed phytates: Nutritional implications. J. Am. Oil Chem. Soc., 56: 736-741.

ETTINGER, M. G. and LUNDEEN, A. J., 1957. First synthesis of a mustard oil glucoside. The enzymatic lossen rearrangement. J. Am. Chem. Soc., 79: 1764-1768.

FAO/UNDP, 1985. Twenty year Fishery Development Plan for Bangladesh. Food and Agricultural Organisation, 1985 (unpublished).

FERRARIS, R. P. and AHEARN, G. A., 1984. Sugar and amino acid transport in fish intestine. Comp. Biochem. Physiol., 77A: 397-413.

FLORENTINO, R. F., VILLAVIEJA, G. M., VALERIO, T. E., DOMDOM, A. C. and RED, E. R., 1985. Fish consumption patterns in the Philippines. ASEAN Food J., 1: 63-69.

FORDIANI, T. R. and KETOLA, H. G., 1980. Effect of treatment on first limiting amino acid of soybean meal in diets of rainbow trout (Salmo gairdneri). Abstract of the 72nd Annual Meeting of the American Society of Animal Science, 27-30 July, Cornell University, Ithaca, New York: p196 (abstract No.222).

FUKUDA, H. and KESAMARU, K., 1972. Studies on the nutritive value of wheat germ as an ingredient of the diets for cultured fishes. III. Improvement of nutritive value of wheat germ containing diets by mixing other feedstuffs. Bull. Jap. Soc. Sci. Fish., 38: 189-194.

FURUICHI, M. and YONE, Y., 1980. Effect of dietary dextrin levels on growth and feed efficiency, and chemical composition of liver, dorsal muscle, and the absorption of dietary protein and dextrin in fishes. Bull. Jap. Soc. Sci. Fish., 46: 225-229.

FURUKAWA, A. and TSUKAHARA, H., 1966. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. Bull. Jap. Soc. Sci. Fish., 32: 502-506.

GLICK, Z. and JOSLYN, M. A., 1970. Food intake depression and other metabolic effects of tannic acid in the rats. J. Nutr., 100: 509-515.

GOERING, K. J., 1963. Obtaining nontoxic protein feed material from mustard seed, rapeseed and similar seeds. US Patent 2,987,399.

GOHL, B., 1981. Tropical Feeds. Food and Agricultural Organisation of the United Nations, Rome. 528pp.

GOSSELIN, R. E. and COGHLAN, E. R., 1953. The stability of complexes between calcium and orthophosphate, polymeric phosphate and phytate. Arch. Biochem. Biophys., 45: 301-304.

GRAF, E., 1983. Applications of phytic acid. J. Am. Oil Chem. Soc., 60: 1861-1867.

HAQUE, K. A., 1985. Freshwater Culture Fisheries of Bangladesh. Paper presented in the National Seminar, 85 on Fisheries Development in Bangladesh, Dhaka, 15-19 January.

HARDY, R. W. and SULLIVAN, C. V., 1983. Canola meal in rainbow trout (Salmo gairdneri) production diets. Can. J. Fish. Aquat. Sci., 40: 281-286.

HARTMAN, G. H., 1979. Removal of phytate from soy protein. J. Am. Oil Chem. Soc., 56: 731-735.

HASAN, M. R., 1986. Husbandry factors affecting survival and growth of carp (Cyprinus carpio L) fry and an evaluation of dietary ingredients available in Bangladesh for the formulation of a carp fry diet. PhD Thesis, Stirling University. 415pp.

HASTINGS, W. H., 1969. Nutritional Score. In: Fish in Research (O. W. Neuhans and J. E. Halver, eds): 263-292. Academic Press, New York.

HEPHER, B., SANDBANK, E. and SHELEF, G., 1979. Alternative protein sources for warm water fish diets. In: Finfish Nutrition and Fish Feed Technology (J. E. Halver and K. Tiews, eds). Vol.I: 328-339, H. Heenemann GmbH, Berlin.

HICKLING, C. F., 1966. On the feeding process in white amur, Ctenopharyngodon idella. J. Zool., 148: 408-419.

HIGGS, D. A., MARKERT, J. R., MACQUARRIE, D. W., MCBRIDE, J. R., DOSANJH, B. S., NICHOLS, C. and HOSKINS, G., 1979. Development of practical dry diets for coho salmon, Oncorhynchus kisutch, using poultry by-product meal, feather meal, soybean and rapeseed meal as major protein sources. In: Finfish Nutrition and Fish Feed Technology (J. E. Halver and K. Tiews, eds). Vol. II: 191-218, H. Heenemann GmbH, Berlin.

HIGGS, D. A., MCBRIDE, J. R., MARKERT, J. R., DOSANJH, B. S., PLOTNIKOFF, M. D. and CLARKE, W. C., 1982. Evaluation of Tower and Candle rapeseed (canola) meal and Bronowski rapeseed protein concentration as potein supplements in practical diets for juvenile chinook salmon (Oncorhynchus tsawytscha). Aquaculture, 29: 1-31.

HILTON, J. W., ATKINSON, J. L. and SLINGER, S. J., 1983. Effect of increased dietary fibre on the growth of rainbow trout (Salmo gairdneri). Can. J. Fish. Aquat. Sci., 40: 81-85.

HILTON, J. W. and SLINGER, S. J., 1986. Digestibility and utilization of canola meal in practical-type diets for rainbow trout (Salmo gairdneri). Can. J. Fish. Aquat. Sci., 43: 1149-1155.

INABA, D., OGINO, C., TAKAMATSU, C., UEDA, T. and KUROKAWA, K., 1963. Digestibility of dietary components in Fishes. II. Digestibility of dietary protein and starch in rainbow trout. Bull. Jap. Soc. Sci. Fish., 29: 242-244.

ISLAM, A., 1983. A report on aquatic culture in Bangladesh. Bgd. Fish Inf. Bull., 1(2): 28pp.

IUPAC-IUB, 1968. Tentative cyclitol nomenclature rules. Eur. J. Biochem., 5: 1.

JACKSON, A. J., CAPPER, B. S. and MATTY, A. J., 1982. Evaluation of some plant proteins in complete diets for the tilapia Sarotherodon mossambicus. Aquaculture, 27: 97-109.

JAUNCEY, K., 1982. The effect of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapis (Sarotherodon mossambicus). Aquaculture, 27: 43-54.

JAUNCEY, K. and ROSS, B., 1982. A Guide to Tilapia Feeds and Feeding. Institute of Aquaculture, University of Stirling, Scotland: 111pp.

JENNER, P. M., HAGAN, E. C., TAYLOR, J. M., TAYLOR, E. L., COOK, E.L. and FITZHUGH, O. G., 1964. Food flavourings and compounds of related structure. I. Acute Oral Toxicity. Fd. Cosmet. Toxicol., 2: 327-343.

JHINGRAN, V. G., 1977. Fish and Fisheries of India. Hindustan Publishing Corporation (India), Jawahar Nager, Delhi, India: 954pp.

JONES, J. D., 1979. Rapeseed protein concentrate preparation and evaluation. J. Am. Oil Chem. Soc., 56: 716-721.

JOSEFSSON, E. and MUNCK, L., 1972. Influence of glucosinolate and a tentative high molecular detrimental factor on the nutritive value of rapeseed meal. J. Sci. Food Agric., 23: 861-868.

JOSEFSSON, E., and MUNCK, L., 1973. Influence of genetically determined difference in glucosinolate content on the value of rapeseed meal. J. Sci. Food Agric., 24: 1265-1271.

JOSEPH, A., 1973. Influence de la technologie traditionelle du manioc sur les teneurs en elements mineraux et en phosphore phytique. Ann. Nutr. Alim., 27: 125-139.

KARIM, M., 1978. Status and Potential of Bangladesh Fisheries. Ministry of Fisheries and Livestock, Govt. of the Peoples Republic of Bangladesh: 125pp.

KAUSHIK, S. J. and LUQUET, P., 1976. Study of the amino acids digestibility of zein diets in rainbow trout. Ann. Hydrobiol., 7: 11-19.

KAUSHIK, S. J. and LUQUET, P., 1980. Influence of bacterial protein incorporation and of sulphur amino acid supplementation to such diets on growth of rainbow trout, Salmo gairdneri Richardson. Aquaculture, 19: 163-175.

KEITH, M. O. and BELL, J. M., 1985. Amino acid supplementation of ammoniated mustard seed meal for use in swine feeds. Can. J. Anim. Sci., 65: 937-944.

KETOLA, H. G., 1982. Amino acid nutrition in fishes: Requirement and supplementation of diets. Comp. Biochem. Physiol., 73B: 17-24.

KIM, Y. K., 1974. Determination of true digestibility of dietary proteins with Cr_2O_3-containing diet. Bull. Jap. Soc. Sci. Fish., 40: 651-654.

KIRCHGESSNER, M., KURZEINGER, H. and SCHWARZ, F. J., 1986. Digestibility of crude nutrients in different feeds and estimation of their energy content for carp (Cyrpinus carpio L). Aquaculture, 58: 185-194.

KITAMIKADO, M., MORISHITA, T. and TACHINO, S., 1964. Digestibility of dietary protein in rainbow trout - II. Effect of starch and oil contents in diets and size of fish. Bull. Jap. Soc. Sci. Fish., 30: 50-54.

KOBAYASHI, T., OTSUKA, S. and YUGARI, Y., 1980. Effect of dietary lysine levels on performance and carcass quality in finishing pigs. Jap. J. Zootech. Sci., 30: 50-54.

KORN, S. and MECEDO, D., 1973. Determination of fat content in fish with a nontoxic nonflammable solvent. J. Fish. Res. Board Can., 30: 1880-1881.

KRATZER, F. H., 1946. The treatment of linseed meal to improve its feeding value for chicks. Poultr. Sci., 25: 541-543.

KRATZER, F. H., 1947. Effect of duration of water treatment on the nutritive value of linseed meal. Poultr. Sci., 26: 90-91.

KRATZER, F. H., 1949. The growth depression of turkey poults caused by linseed oil meal. Poultr. Sci., 28: 618-620.

KRATZER, F. H., ALLRED, J. B., DAVIS, P. N., MARSHALL, B. J. and VOHRA, P., 1959. The effect of autoclaving soybean protein and the addition of ethylenediaminetetracetic acid on the biological availability of dietary zinc for turkey poults. J. Nutr., 68: 313-322.

KRATZER, F. H. and WILLIAMS, D. E., 1948. The relation of pyridoxine to the growth of chicks fed rations containing linseed oil meal. J. Nutr., 36: 297-305.

KRATZER, F. H., WILLIAMS, D. E., MARSHALL, B. and DAVIS, P. N., 1954. Some properties of the chick growth inhibitor in linseed oil meal. J. Nutr., 52: 555-563.

LAKSHMANAN, M. A. V., MURTY, D. S., PILLAI, K. K. and BANERJEE, S. C., 1967. On a new artificial feed for carp fry. FAO Fish. Rep., 44: 373-387.

LALL, S. P., 1979. Minerals in Finfish Nutrition. In: Finfish Nutrition and Fish Feed Technology (J. E. Halver and K. Tiews, eds). Vol.I: 85-97. H. Heenemann GmbH, Berlin.

LANGER, P. and STOLE, V., 1965. Goitrogenic activity of allyl isothiocyanate - a widespread natural mustard oil. Endocrinology, 76: 151-155.

LAW, A. T., 1984. Nutritional study of jelawat, Leptobarbus hoevenii (Bleeker), fed on pelleted feed. Aquaculture, 41: 227-233.

LAW, A. T., 1986. Digestibility of low-cost ingredients in pelleted feed by grass carp (Ctenopharyngodon idella). Aquaculture, 51: 97-103.

LEASE, J. G., 1966. The effect of autoclaving sesame meal on its phytic acid content and on the availability of its zinc to chick. Poult. Sci., 45: 237-241.

LEASE, J. G., BARNETT, D., LEASE, E. J. and TURK, D. E., 1960. The biological unavailability to the chick of zinc in a sesame meal ration. Poult. Sci., 39: 1269-1272.

LIENER, I. E., 1975. Endogenous toxic factors in oilseeds residue. In: D. Halliday (ed) Proc. Conf. Anim. Feed Trop. Sub-Trop. Origin. Tropical Products Institute, London: 179-188.

LIENER, I. E., 1977. Removal of naturally occurring toxicants. Adv. Chem. Ser., 160: 293-300.

LIENER, I. E., 1980. Miscellaneous toxic factors. In: I. E. Liener (ed) Toxic constituents of plant foodstuffs. Academic Press, New York: 429-467.

LIKUSKI, H. J. A. and FORBES, R. M., 1965. Mineral utilization in the rats. IV. Effects of calcium and phytic acid on the utilization of dietary zinc. J. Nutr., 85: 230-234.

LO, M. T. and HILL, D. C., 1971. Effect of feeding a high level of rapeseed meal on weight gain and thyroid function of rats. J. Nutr., 101: 975-980.

LO, M. T. and BELL, J. M., 1972. Effects of various dietary glucosinolate on growth, feed intake and thyroid function of rats. Can. J. Anim. Sci., 52: 295-298.

LOVELL, R. T., 1977. Digestibility of nutrients in feedstuffs for catfish. In: R. R. Stickney and R. T. Lovell (eds), Nutrition and Feeding of Channel Catfish. Southern Cooperative Series Bull. 218, Auburn University, Auburn, Alabama: 33-37.

LYSAK, A. W., 1979. Relationship between the thyroid localization and growth rate in young carp (Cyprinus carpio L) in different feeding conditions. In: Finfish Nutrition and Fish Feed Technology (J. E. Halver and K. Tiews, eds), Vol.I: 231-239, H. Heenemann GmbH, Berlin.

MACGREGOR, H. I. and MCGINNIS, J., 1948. Toxicity of linseed meal for chicks. Poult. Sci., 27: 141-145.

MADHUSUDHAN, K. T. and SINGH, N., 1983. Studies on linseed proteins. J. Agric. Food Chem., 31: 959-963.

MAHESWARI, P. N., STANLEY, D. W. and GRAY, J. I., 1981. Detoxification of rapeseed products. J. Food Proct., 44: 459-470.

MAHNKEN, C. V. W., SPINELLI, J. and WAKNITZ, F. W., 1980. Evaluation of an alkaline yeast (Candida sp) as a substitute for fish meal in Oregon Moist Pellet; feeding trials with coho salmon (Oncorhynchus kisutch) and rainbow trout (Salmo gairdneri). Aquaculture, 20: 41-56.

MANDOKHOT, V. M. and SINGH, N., 1979. Studies on linseed (Linum usitatissimum) as a protein source for poultry. I. Processing of demucilaging and dehulling of linseed and evaluation of processed materials by chemical analysis and with rats and chicks. J. Food Sci. Tech., 16: 25-31.

MANI, K. V., NIKOLAICZUK, N. and MAW, W. A., 1949. Flaxseed mucilage and its effect on the feeding value of linseed oil meal in chick rations. Sci. Ag., 29: 86-90.

MARANGOS, A. and HILL, R., 1974. The hydrolysis and absorption of thioglucosides of rapeseed meal. Proc. Nutr. Soc., 33: 90A.

MARQUARDT, R. R., WARD, A. T., CAMPBELL, L. D. and CANSFIELD, P. E., 1977. Purification, identification and characterization of a growth inhibitor in faba beans (Vicia faba L var. minor). J. Nutr., 107: 1313-1316.

MAYNARD, L. A. and LOOSLI, J. K. (eds), 1969. Animal Nutrition. 6th edition. McGraw Hill Book Company: 613pp.

MCDONALD, P., EDWARDS, R. A. and GREENHALGH, J. F. D., 1981. Animal Nutrition. Longman, London and New York: 479pp.

MCGINNIS, J. and POLIS, H. L., 1946. Factors affecting the nutritive value of linseed meal for growing chicks. Poult. Sci., 25: 408-410.

MESKE, C. and PFEFFER, E., 1978. Tests on micro-algae, yeast and casein as components of a fish meal-free dry food for carp. In: Animal Research and Development, Vol.7, Tubingen, Institute for Scientific Cooperation: 112-121.

MILLER, D. S. and BENDER, A. E., 1955. The determination of the net utilization of proteins by a shortened method. Br. J. Nutr., 9: 382-388.

MOKADY, S., YANNAI, S., EINAV, P. and BERK, Z., 1980. Protein nutritive value of several micro-algae species for young chickens and rats. In: Algal Biomass. (G. Shelef and C. J. Soeder, eds). Elsevier/North Holland Biomedical Press: 656-660.

MONTGOMERY, R. D., 1964. Observations on the cyanide content and toxicity of tropical pulses. West Indian Med. J., 13: 1-11.

MORRIS, E. R. and ELLIS, R., 1980. Effect of dietary phytate/zinc molar ratio on growth and bone zinc response of rats fed semi-purified diets. J. Nutr., 110: 1037-1045.

MUKHERJEE, K. D., AFZALPURKAR, A. B. and EL-NOCKRASHY, A. S., 1976. Production of low glucosinolate rapeseed meals. Fette, Seifen, Anstrichm., 78: 306-311.

MURAI, T., AKIYAMA, T. and NOSE, T., 1981. Use of crystalline amino acids coated with casein in diets for carp. Bull. Jap. Soc. Sci. Fish., 47: 523-527.

MURAI, T., AKIYAMA, T. and NOSE, T., 1982a. Effects of casein coating on utilization of dietary amino acid by fingerling carp and channel catfish. Bull. Jap. Soc. Sci. Fish., 48: 787-792.

MURAI, T., OGATA, H. and NOSE, T., 1982b. Methionine coated with various materials supplemented to soybean meal diet for fingerling carp, Cyrpinus carpio and channel catfish, Ictalurus punctatus. Bull. Jap. Soc. Sci. Fish., 48: 85-88.

MURAI, T., OGATA, H., KOSUTARAK, P. and ARAI, S., 1986. Effects of amino acid supplementation and methanol treatment on utilization of soy flour by fingerling carp. Aquaculture, 56: 197-206.

MUSTAKAS, G. C., KIRK, L. D. and GRIFFIN, E. I., Jr., 1962. Mustard seed processing: Bland protein meal, bland oil, and allyl isothiocyanate as a by-product. J. Am. Oil Chem. Soc., 39: 372-375.

MUSTAKAS, G. C., GRIFFINS, E. L., Jr., GASTROCK, E. A., D'AQUIN, E.L., KEATING, E. J. and POTTON, E. L., 1963. Enzymatic process for mustard seed to produce oil, meal, and allyl isothiocyanate. Biotech. Bioeng., 5: 27-39.

MUSTAKAS, G. C., KIRK, L. D., SHONS, V. E. and GRIFFIN, E. L., Jr., 1965. Mustard seed processing: Improved methods for isolating the pungent factor and controlling protein quality. J. Am. Oil Chem. Soc., 42: 33-37.

NATIONAL RESEARCH COUNCIL, 1977. Nutrient Requirements of Warmwater Fishes. National Academy of Sciences, Washington DC: 78pp.

NATIONAL RESEARCH COUNCIL, 1983. Nutrient Requirements of Warmwater Fishes and Shellfishes. National Academy of Sciences, Washington DC: 102pp.

NEUBERG, C., 1908. Zur Frageder Konstitution des "Phytens". Biochem. Z., 9: 557-559.

NEW, M. B. and SINGHOLKA, S., 1982. Freshwater prawn farming. A manual for the culture of Macrobrachium rosenbergii. FAO Fish Tech.Pap., 225: 116pp.

NISHIE, K. and DAXENBICHLER, M. E., 1980. Toxicology of glucosinolates, related compounds (Nitriles, R-goitrin, isothiocyanates) and vitamin U found in cruciferae. Fd. Cosmet. Toxicol., 18: 159-172.

NOSE, T., 1967. On the metabolic faecal nitrogen in young rainbow trout. Bull. Freshwater Fish. Res. Lab. (Tokyo), 17: 97-105.

NOSE, T., 1974. Effect of amino acids supplement to petroleum yeast on growth of rainbow trout fingerlings - I. A preliminary experiment. Bull. Freshwater Fish. Res. Lab. (Tokyo), 21: 57-60.

OBERLEAS, D., MUHRER, M. E. and O'DELL, B. L., 1962. Effects of phytic acid on zinc availability and parakeratosis in swine. J. Anim. Sci., 21: 57-60.

OBERLEAS, D., MUHRER, M. E. and O'DELL, B. L., 1966. Dietary metal-complexing agents and zinc availability in the rats. J. Nutr., 90: 56-62.

OBERLEAS, D., 1973. "Phytates". In: Toxicants Occurring Naturally in Foods. National Academy of Science, Washington DC: 363-371.

O'DELL, B. L., NEBERNE, P. M. and SAVAGE, J. E., 1958. Significance of dietary zinc for the growing chicken. J. Nutr., 65: 503-524.

O'DELL, B. L. and SAVAGE, J. E., 1960. Effect of phytic acid on zinc availability. Proc. Soc. Exp. Biol. Med., 103: 304-306.

O'DELL, B. L., 1962. Mineral availability and metal-binding constituents of the diet. Proc. Cornell Nutr. Conf.: 77-83.

O'DELL, B. L., YOHE, J. M. and SAVAGE, J. E., 1964. Zinc availability in the chick as affected by phytate, calcium and ethylenediaminetetracete. Poult. Sci., 43: 15-19.

OGATA, H., ARAI, S. and NOSE, T., 1983. Growth responses of chevy salmon Oncorhynchus masou and diets supplemented with amino acids. Bull. Jap. Soc. Sci. Fish., 49: 1381-1385.

OGINO, C., 1980. Requirements of carp and rainbow trout for essential amino acids. Bull. Jap. Soc. Sci. Fish., 46: 171-174.

OGINO, C. and SAITO, K., 1970. Protein nutrition in fish - I. The utilization of dietary protein by young carp. Bull, Jap. Soc. Sci. Fish., 36: 250-254.

OGINO, C. and CHEN, M. S., 1973. Protein nutrition in fish - III. Apparent and true digestibility of dietary protein in carp. Bull. Jap. Soc. Sci. Fish., 39: 649-651.

OGINO, C., KAKINO, J. and CHEN, M. S., 1973. Protein nutrition in fish. II. Determination of metabolic fecal nitrogen and endogenous nitrogen excretions of carp. Bull. Jap. Soc. Sci. Fish., 39: 519-523.

OLIVER, S. L., MCDONALD, B. E. and OPUSZYNSKA, T., 1971. Weight gain, protein utilization, and liver histochemistry of rats fed low and high thioglucoside content rapeseed meals. Can. J. Physiol. Pharmacol., 49: 448-458.

OLSEN, O. and SORENSEN, H., 1981. Recent advances in the analysis of glucosinolates. J. Am. Oil Chem. Soc., 58: 857-865.

OLUKUNLE, O. A., 1982. Partial plant protein substitution in diets of first feeding stage fry of tilapia: Oreochromis (=Sarotherodon) mossambicus. MSc Thesis, University of Stirling: 55pp.

PANTHA, M. B., 1982. The use of soybean in practical feeds for tilapia (Oreochromis niloticus). MSc Thesis, University of Stirling: 60pp.

PFEFFER, E., 1982. Utilization of dietary protein by salmonid fish. Comp. Biochem. Physiol., 73B: 51-57.

PILLAY, T. V. R., 1981. State of Aquaculture 1981. Paper presented at the World Conference in Aquaculture and International Aquaculture Trade Show, 21-25 September 1981, Venice: 17pp.

PLAKAS, S. M. and KATAYAMA, T., 1981. Apparent digestibilities of amino acids from three regions of the gastro-intestinal tract of carps (Cyrpinus carpio) after ingestion of a protein and corresponding free amino acid diet. Aquaculture, 24: 309-314.

POST, G., SHANKS, W. E. and SMITH, R. R., 1965. A method for collecting metabolic excretion from fish. Progr. Fish. Cult., 27: 108-111.

POSTON, H. A., RIIS, R. C., RUMSEY, G. L. and KETOLA, H. G., 1977. The effect of supplemental dietary amino acids, minerals and vitamins on salmonids fed cataractogenic diets. Cornell Vet., 67: 472-509.

PRICE, M. L., BUTLER, L. G., FEATHERSTONE, W. R. and ROGLER, J. C., 1978. Detoxification of high tannin sorghum grain. Nutr. Rep. Int., 17: 229-236.

RAPA, 1985. Food Consumption in Asia-Pacific Region 1972-1982. RAPA Monogr. 1985/9: 29pp.

RAVENHOLT, A., 1982. Malnutrition in the Philippines. UFSI Rep. Southeast Asia Ser. 20: 73-84.

RAYMOND, W. D., JOJO, W. and NICODEMUS, Z., 1941. The nutritive value of some Tanganyikan foods - II. Casava. E. Afr. Agric. J., 6: 154-159.

REYNOLDS, J. R. and YOUNGS, C. G., 1964. Effect of seed preparation on efficiency and oil quality in filtration extraction of rapeseed. J. Am. Oil Chem. Soc., 41: 63-65.

RICHARDS, A., 1983. The potential of sunflower and sesame meals as alternative source of protein in diets for Oreochromis niloticus. MSc Thesis, Institute of Aquaculture, University of Stirling: 82pp.

RICHARDSON, N. L., HIGGS, D. A., BEAMES, R. M. and MCBRIDGE, J. B., 1985. Influence of dietary calcium, phosphorous, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile chinook salmon (Oncorhynchus tshawytscha). J. Nutr., 115: 553-567.

ROACH, A. G., SANDERSON, A. and WILLIAMS, D. R., 1967. Comparison of methods for the determination of available lysine value in animal and vegetable protein sources. J. Sci. Food. Agric., 18: 274-278.

ROBINSON, E. H., WILSON, R. P. and POE, W. E., 1980. Re-evaluation of the lysine requirement and lysine utilization by fingerling channel catfish. J. Nutr., 110: 2313-2316.

ROBINSON, E. H., RAWLES, S. D., OLDENBURGH, P. W. and STICKNEY, R. R., 1984. Effects of feeding glandless or glanded cottonseed products and gossypol to Tilapia aurea. Aquaculture, 38: 145-154.

ROSS, L. G. and GEDDES, J. A., 1979. Sedation of warm water fish species in aquaculture research. Aquaculture, 16: 183-186.

RUMSEY, G. L. and KETOLA, H. G., 1975. Practical diet for Atlantic salmon (Salmo salar) fry and fingerlings. Prog. Fish Cult., 37: 253-256.

RUTKOWSKI, A., 1970. Effect of processing on the chemical composition of rapeseed meal. Proc. Int. Conf. Science, Technology and Marketing of Rapeseed Products, Quebec, Canada, 1974: 496pp. Rapeseed Association of Canada and the Department of Industry, Trade and Commerce, Ottawa, Canada.

RYAN, T. A., Jr., JOINER, B. L. and RAYAN, B. R., 1976. Minitab Student Handbook. Wadsworth Publishing Co Inc, Belmont, California.

RYCHLY, J. and SPANNHOF, L., 1979. Nitrogen balance in trout - I. Digestibility of diets containing varying levels of protein and carbohydrate. Aquaculture, 16: 39-46.

SALUNKHE, D. K. and WU, M. T., 1977. Toxicants in plants and plant products. CRC Crit. Rev. Food Sci. Nutr., 9: 265-324.

SCHMITZ, O., GREUEL, E. and PFEFFER, E., 1983. A method for determining digestibility of nutrients in eels. Aquaculture, 32: 71-78.

SCHWARZ, F. J., KURZINGER, H. and KIRCHGESSNER, M., 1986. Zur Verdaulichkeit des Rohproteins von Blutmehl, kartoffeleiweiss und Bitterlupinen in Mischfuttermitteln fur karfen (Cyprinus carpio). Bayer Landwirtsch, Johrb., 63: 595-602.

SEN, P. R., RAO, N. G. S., GOSH, S. R. and ROUT, M., 1978. Observation on the protein and carbohydrate requirements of carps. Aquaculture, 13: 245-255.

SHAH, E. H., NIAZI, A. H. K., ALI, S. and MAHMOOD, I., 1977. Detoxification of mustard seed cake. Pakistan J. Sci. Ind. Res., 20: 316-320.

SHIAU, S. Y., CHUANG, J. L. and SUN, C. L., 1987. Inclusion of soybean meal in tilapia (Oreochromus niloticus x O. aureus) diets at two protein levels. Aquaculture, 65: 251-261.

SIN, A. W., 1973. The utilization of dietary protein for growth of young carp (Cyrprinus carpio) in relation to variation in fat intake. Hong Kong Fish. Bull., 3: 83-88.

SINGH, K. and ARORA, S. P., 1978. Studies on detanning and determining the nutritive value of alkali-treated salseed meal (Shorea robusta). Indian J. Anim. Sci., 48: 84-89.

SINGH, N. and PUNIA, M. P., 1979. Linseed cake in broiler rations. Poult. Guide, Vol. August: 39-42.

SINGLETON, V. L. and KRATZER, F. H., 1973. Plant Phenolics: 309-345 In: Toxicants occurring naturally in foods. Nat. Acad. Sci., Washington DC.

SKREDE, A., 1979. Utilization of fish and animal by-products in mink nutrition - IV. Faecal excretion and digestibility of nitrogen and amino acids by mink fed cod (Gadus morrhua) fillet or meat and bone meal. Acta Agric. Scand., 29: 241-257.

SKREDE, A., KROGDAHL, A. and AUSTRENG, E., 1980. Digestibility of amino acids in raw fish flesh and meat and bone meal for the chicken, fox, mink and rainbow trout. Z. Tierphysiol. Tierernahrg, u. Futtermittelkde., 43: 92-101.

SLINGER, S. J., 1977. Improving nutritional properties of rapeseed. J. Am. Oil Chem. Soc., 54: 94A-99A.

SLUMP, P., VANBEEK, L., JANSSEN, W. M. M. A., TERPSTRA, K., LENIS, N.P. and SMITS, B., 1977. A comparative study with pigs, poultry and rats of the amino acid digestibility of diets containing crude protein with diverging digestibilities. Z. Tierphysiol. Tierernahrg. Futtermittelkde., 39: 257-272.

SMITH, B. W. and LOVELL, R. T., 1973. Determination of apparent digestibility in feeds for channel catfish. Trans. Am. Fish. Soc., 102: 831-835.

SMITH, R. R., 1971. A method for determining the metabolizable energy of fish feeds. Prog. Fish Cult., 33: 132-136.

SMITH, R. R., PETERSON, M. C. and ALLRED, A. C., 1980. Effect of leaching on apparent digestion coefficients of feedstuffs for salmonids. Prog. Fish Cult., 42: 195-199.

SOARES, J. H., MILLER, D., FITZ, N. and SANDERS, M., 1971. Some factors affecting the biological availability of amino acids in fish protein. Poult. Sci., 50: 1134-1143.

SPINELLI, J., HOULE, C. R. and WEKELL, J. C., 1983. The effect of phytates on the growth of rainbow trout (Salmo gairdneri) fed purified diets containing varying quantities of calcium and magnesium. Aquaculture, 30: 71-83.

SPINELLI, J., MAHNKEN, C. and STENBERG, M., 1979. Alternative sources of proteins for fish meal in salmonid diets. In: Finfish Nutrition and Fish Feed Technology (J. E. Halver and K. Tiews, eds), Vol.II: 131-142. H. Heenemann GmbH, Berlin.

SRIVASTAVA, V. K. and HILL, D. C., 1976. Effect of mild heat treatment on the nutritive value of low glucosinolate low erucic acid rapeseed meals. J. Sc. Food Agric., 27: 953-958.

SRIVASTAVA, V. K., PHILBRICK, D. J. and HILL, D. C., 1975. Response of rats and chicks to rapeseed subjected to different enzymatic treatments. Can. J. Anim. Sci., 55: 331-335.

STIRLING, H. P., 1985. Chemical and biological methods of water analysis for aquaculturists. Institute of Aquaculture, University of Stirling: 119pp.

SWAMINATHAN, M., 1967. Availability of plant proteins. In: A. A. Albanese (ed) Newer Methods of Nutritional Biochemistry, Vol. III. Academic Press, New York: 197-241.

TACON, A. G. J., 1981. The possible substitution of fish meal in fish diets. Proceedings of the Scottish Marine Biological Association and Highlands and Islands Development Board fish farm meeting, Oban, Scotland, 26-27 February 1981: 46-56.

TACON, A. G. J. and FERN, P. N., 1976. Use of activated sewage in trout diets. Nutr. Rep. Int., 13: 549-561.

TACON, A. G. J. and DE SILVA, S. S., 1983. Mineral composition of some commercial fish feeds available in Europe. Aquaculture, 31: 11-20.

TACON, A. G. J., STAFFORD, E. A. and EDWARDS, C. A., 1983a. A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. Aquaculture, 35: 187-199.

TACON, A. G. J., HUASTER, J. V., FEATHERSTONE, P. B., KERR, K. and JACKSON, A. J., 1983b. Studies on the utilization of full fat soybean and solvent extracted soybean meal in a complete diet for rainbow trout. Bull. Jap. Soc. Sci. Fish., 49: 1437-1443.

TACON, A. G. J., JAUNCEY, K., FALAYE, A., PANTHA, M., MACGOWAN, I., and STAFFORD, E. A., 1984. The use of meat and bone meal, hydrolysed feathermeal and soybean meal in practical fry and fingerling diets for Oreochromis niloticus. In: Proceedings of First International Symposium on Tilapia in Aquaculture (L. Fishelson and Z Yaron, eds): 356-365. Tel Aviv University Press, Israel.

TACON, A. G. J. and RODRIGUES, A. M. P., 1984. Comparison of chromic oxide, crude fibre, polyethylene and acid insoluble ash as dietary markers for the estimation of apparent digestibility coefficients in rainbow trout. Aquaculture, 43: 391-399.

TAKEUCHI, T., WATANABE, T. and OGINO, C. 1979. Digestibility of hydrogenated fish oils in carp and rainbow trout. Bull. Jap. Soc. Sci. Fish., 45: 1521-1526.

TAKEUCHI, T., YOKOYAMA, M., WATANABE, T. and OGINO, C., 1978. Optimum ratio of dietary energy to protein for rainbow trout. Bull. Jap. Soc. Sci. Fish., 44: 729-732.

TFYP, (1985-90). The Third Five Year Plan. Planning Commission, Ministry of Planning, Government of the Peoples Republic of Bangladesh, Dhaka, December 1985.

TIEWS, K., KOOPS, H., GROPPS, J. and BECK, H., 1979. Compilation of fish meal-free diets obtained in rainbow trout (Salmo gairdneri) feeding experiments at Hamburg (1970-1977/78). In: Finfish Nutrition and Fish Feed Technology (J. E. Halver and K. Tiews, eds), Vol. II: 219-228, H. Heenemann GmbH, Berlin.

TOOKY, H. L., VAN ETTEN, C. H. and DAXENBICHLER, M. E., 1980. Glucosinolates. In: I. E. Liener (ed) Toxic Constituent of Plant Foodstuffs. Academic Press, New York: 103-142.

TUNISON, A. V. and MCCAY, C. M., 1938. Linseed meal and the removal of its toxic properties. The nutrition of trout. Cortland Hatch Rep. No.6, State of New York Conservation Department, Albany.

UFODIKE, E. B. C. and MATTY, A. J., 1983. Growth responses and nutrient digestibility in mirror carp (Cyrpinus carpio) fed different levels of cassava and rice. Aquaculture, 31: 41-50.

VALAER, P., 1941. Report on whisky and rum. Tannins in potable spirits. J. Assoc. Off. Agric. Chem., 24: 224-227.

VAN ETTEN, C. H., 1969. Goitrogen: 102. In: I. E. Liener (ed) Toxic Constitutents in Plant Feedstuffs. Academic Press, New York.

VAN ETTEN, C. H., DAXENBICHLER, M. E., PETER, J. E. and TOOKEY, H. L.. 1966. Variation in enzymatic degradation products from the major thioglucosides in Crambe abyssinica and Brassica napus seed meals. J. Agric. Food Chem., 14: 426-430.

VAN ETTEN, C. H. and WOLFF, I. A., 1973. Natural sulfur compounds: 210-234. In: Toxicans Occurring Naturally in Foods. National Academy of Science, Washing DC.

VELU, J. G., BAKER, D. H. and SCOTT, H. M., 1971. Protein and energy utilization by chicks fed graded levels of a balanced mixture of crystalline amino acids. J. Nutr., 101: 1249-1256.

VENS-CAPPELL, B., 1984. The effects of extrusion and pelleting of feed for trout on the digestibility of protein, amino acids and energy and feed conversion. Aquacultural Engineering, 3: 71-89.

VIOLA, S., MOKADY, S., RAPPAPORT, U. and ARIELI, Y., 1982. Partial and complete replacement of fish meal by soybean meal in feeds for intensive culture of carp. Aquaculture, 26: 223-226.

VOHRA, P., KRATZER, F. H. and JOSLYN, M. A., 1966. The growth depressing and toxic effects of tannins to chicks. Poult. Sci., 46: 135-142.

WELCH, R. M. and HOUSE, W. A., 1982. Availability of rats of zinc from soybean seeds as affected by maturing of seed, source of dietary protein and soluble phytate. J. Nutr., 112: 879-885.

WETHLI, E., MORRIS, T. R. and SHRESTA, T. P., 1975. The effect of feeding high levels of low quality proteins to growing chickens. Br. J. Nutr., 34: 363-373.

WETTER, L. R., 1955. The determination of mustard oils in rapeseed meal. Can. J. Biochem. Physiol., 33: 980-984.

WHEELER, E. L. and FERREL, R. E., 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chem., 48: 312-320.

WILSON, R. P. and POE, W. E., 1985. Apparent digestible protein and energy coefficients of common feed ingredients for channel catfish. Prog. Fish Cult., 47: 154-158.

WILSON, R. P., ROBINSON, E. H. and POE, W. E., 1981. Apparent and true availability of amino acids from common feed ingredients for channel catfish. J. Nutr., 111: 923-929.

WINDELL, J. T., FOLTZ, J. W. and SAROKON, J. A., 1978a. Methods of faecal collection and nutrient leaching in digestibility studies. Prog. Fish. Cult., 40: 51-55.

WINDELL, J. T., FOLTZ, J. W. and SAROKON, J. A., 1978b. Effect of fish size, temperature and amount fed on nutrient digestibility of a pelleted diet by rainbow trout, Salmo gairdneri. Trans. Am. Fish Soc., 107: 613-616.

YAMADA, S., TANAKA, Y. and KATAYAMA, T., 1981. Feeding experiments with carp fry fed on amino acid diet by increasing the number of feedings per day. Bull. Jap. Soc. Sci. Fish., 47: 1247.

YAPAR, Z. and CLANDININ, D. R., 1972. Effect of tannins in rapeseed meal on its nutritional value for chicks. Poult. Sci., 51: 222-228.

YURKOWSKI, M., BAILEY, J. K., EVANS, R. E., TABACHEK, J. A. L., AYLES, G. B. and EALES, J. G., 1978. Acceptability of rapeseed proteins in diets of rainbow trout (Salmo gairdneri). J. Fish. Res. Board. Can., 35: 951-962.

ZEITLER, M. H., KIRCHGESSNER, M. and SCHWARZ, F. J., 1984. Effect of different protein and energy supplies on carcass composition of carp (Cyprinus carpio). Aquaculture, 36: 37-48.